A STUDY ON THE MYCOLOGICAL PROFILE, CATEGORIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CHRONIC FUNGAL RHINOSINUSITIS IN A TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON THE MYCOLOGICAL PROFILE, CATEGORIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CHRONIC FUNGAL RHINOSINUSITIS IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR. K.KAVITHA,** during the period of her Post graduate study from June 2009 to May 2012 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for **M.D. MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R. Medical University to be held in April 2012.

Dr.V.Kanagasabai, M.D.,
Dean,
Madras Medical College &
Rajiv Gandhi Government General Hospital,
Chennai – 600 003

Dr.R.Manjula,M.D.,
Director , Institute of Microbiology,
Madras Medical College &
Rajiv Gandhi Government General Hospital,
Chennai – 600 003

DECLARATION

I declare that the dissertation entitled **"A STUDY ON THE MYCOLOGICAL PROFILE, CATEGORIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CHRONIC FUNGAL RHINOSINUSITIS IN A TERTIARY CARE HOSPITAL"** submitted by me for the degree of M.D. is the record work carried out by me during the period of **January 2010 to June 2011** under the guidance of Professor **Dr.G.JAYALAKSHMI M.D., DTCD.,** Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Microbiology (Branch IV) examination to be held in May 2012.

Place: Chennai
Date :

Signature of the Candidate
(Dr.K.KAVITHA)

Signature of the Guide
Prof .Dr.G.JAYALAKSHMI, MD., DTCD.,
Professor,
Institute of Microbiology,
Madras Medical College,
Chennai-3

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CONTENTS

INTRODUCTION

Rhinosinusitis is defined as the inflammation of nasal and paranasal sinus mucosa and is associated with mucosal alterations ranging from inflammatory thickening to gross nasal polyp formation. Rhinosinusitis is a common disorder affecting approximately 20% of the population at some time of their lives. It has been estimated to affect approximately 23 million patients (4% of adult population)in the United States each year[6]. A recent survey reported that 11% of adults recalled a health professional's diagnosis of sinusitis.[1]

The International Classification of Diseases divides Rhinosinusitis into acute and chronic forms according to the duration of symptoms. Acute rhinosinusitis(ARS) lasts upto 12 weeks with complete resolution of symptoms, whereas Chronic Rhinosinusitis (CRS) persists beyond 12 weeks.[1,83,84]

The inflammation of the nasal and sinus mucosa may be due to microorganisms (bacteria and fungi), allergic and non allergic immunological inflammation, and noninfectious, non immunological causes. The subset of rhinosinusitis cases where the etiological role of fungi is proven or is considered to be important (due to its isolation from tissue biopsy samples) is referred to as fungal rhinosinusitis (FRS). Fungal sinusitis is being increasingly recognized in persons of all age groups, resulting in great socioeconomic effects. Previously, 5-15% of cases of chronic rhino sinusitis cases were

thought to be of fungal etiology. However, after the claim of fungus to be the etiological agent in majority of cases of CRS by Ponikau et al, 1999, the impact of fungal rhinosinusitis seems to be tremendous. [5]

Fungal rhinosinusitis can range from the benign localized fungal colonisation to the extremely aggressive acute invasive form having a very broad spectrum of disease. Fungal sinusitis causes significant physical symptoms, severe quality of life impairment, and can substantially impair daily functioning. The economic effect is also huge. As the incidence of chronic fungal rhinosinusitis has increased over the last decade, the economic effect is expected to be more. The patients have high morbidity and even may have high mortality especially those having acute invasive fungal rhinosinusitis. [22]

Our knowledge about the epidemiology and medical mycology of the disease remains incomplete and subject to newer findings and research despite recognition of FRS as a serious disease entity for more than two centuries, The disease is often neglected and misdiagnosed especially in developing countries like India, where FRS is one among the neglected diseases. Few studies have been done to quantitate the impact of fungi in the pathogenesis of sinusitis in India and fewer in Tamilnadu. This study was conducted in a tertiary care hospital to evaluate the occurrence of fungi as etiology for the occurrence of rhinosinusitis in patients admitted with a radiological diagnosis of

rhinosinusitis and undergoing diagnostic and therapeutic endoscopic procedures for the same.

Resistance to antifungals is not that commonly encountered problem .It is an emerging concern as resistance of *Aspergillus spp* to standard antifungals have been noted and reported. Hence, anti fungal susceptibility testing is advised for isolates causing FRS, particularly for invasive forms, chronic granulomatous forms and those occurring in immunocompromised. Anti fungal susceptibility testing is not as simple as that of bacteria. It is tedious and costly and not routinely attempted in all laboratories .So, an attempt has been made in this study to try and compare different methods of susceptibility testing for filamentous fungi.

AIM OF THE STUDY

- To isolate the fungi causing chronic fungal rhinosinusitis.
- To identify and speciate the fungi.
- To categorise the types of fungal sinusitis.
- To assess the risk factors favouring fungal involvement of paranasal sinuses.
- To study the susceptibility pattern of the fungal isolates to standard anti fungal drugs.
- To compare different methods of susceptibility testing for the fungal isolates.

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REVIEW OF LITERATURE

HISTORICAL PERSPECTIVES

The identification and documentation of fungus as a cause of chronic rhinosinusitis in patients goes way back to the 18th century when Plaignaud in 1791 described 'fungus tumor' in the maxillary sinus of a 22-year-old soldier[6]. Then slowly fungus has gained importance as a common cause of sinusitis and various people have documented fungi in sinusitis. In a controversial article, Ponikau et al, 1999, using novel diagnostic techniques, demonstrated the presence of fungi and eosinophils in 96% of chronic fungal sinusitis .[5]If their findings are true, this will effectively mean that nearly all patients of CRS have a fungal etiology.

CATEGORISATION OF FUNGAL SINUSITIS

Though a lot of controversies surround the categorization of FRS, most commonly accepted system divides FRS into two categories : Invasive and noninvasive depending on the invasion of fungi across mucous membrane.

Invasive FRS is subcategorized as into three groups: acute invasive (fulminant), granulomatous invasive, and chronic invasive. Noninvasive FRS is also further subcategorised as into three groups: Localized colonization, fungal ball (sinus mycetoma), and eosinophil related FRS (including allergic fungal rhinosinusitis, eosinophilic fungal rhinosinusitis).[6]

1. INVASIVE FUNGAL RHINOSINUSITIS

A. ACUTE INVASIVE (FULMINANT) FRS:

Commonly caused by members of the class *Zygomycetes* or by *Aspergillus* spp. This disease occurs more often in the immunocompromised patients,[22,25,27] and associated with a mortality rate exceeding 50%. The disease is characterized by a predominant vascular invasion .[7]

B .GRANULOMATOUS INVASIVE FRS:

This disease has been described primarily in Sudan, India, Pakistan and Saudi Arabia, and rarely in the United States, and is characterized by a time course of more than 12 weeks.[7,9,56] The entity presents with an enlarging mass in the cheek, orbit, nose, and paranasal sinuses in immunocompetent hosts.[88]

3. CHRONIC INVASIVE FRS:

Chronic invasive FRS is a slowly destructive process that most commonly affects the ethmoid and sphenoid sinuses but may involve any paranasal sinus. The entity is usually seen in the patients having diabetes mellitus or on corticosteroid treatment.[55,56,57,89] Cultures of tissue are positive in >50% of cases and *A. fumigatus* is the most common agent isolated[6].

2. NONINVASIVE FRS:

A. LOCALIZED FUNGAL COLONIZATION*:*

This disease entity refers to the asymptomatic colonization of mucous crusts within the nasal cavity by fungi, often in patients who had previous sinus surgery.[10]

B. SINUS FUNGAL BALL/ MYCETOMA/ASPERGILLOMA OF SINUSES*:*

Sinus fungal ball is described as the presence of noninvasive accumulation of dense conglomeration of fungal hyphae in one sinus cavity[95], usually the maxillary sinus, though the disease may affect other sinuses or rarely multiple sinuses.[11,90]The disease is defined by the following criteria: Radiological evidence of sinus opacification with or without radiographic heterogenecity, mucopurulent cheesy or clay-like materials within the sinus, a dense conglomeration of hyphae separate from the sinus mucosa ,nonspecific chronic inflammation of the mucosa, no predominance of eosinophils or granuloma or allergic mucin, no histopathological evidence of fungal invasion of mucosa.[11,90]

C. *EOSINOPHIL RELATED FRS:*

i. ALLERGIC FUNGAL RHINOSINUSITIS (AFRS):

Bent and Kuhn proposed five diagnostic criteria for the entity of AFRS: **Type I hypersensitivity, nasal polyposis, characteristic findings on CT**

scan, presence of fungi on direct microscopy or culture, and allergic mucin containing fungal elements without tissue invasion.[4] The 'peanut-butter' or'cottage-cheese' like mucin evacuated from sinuses of patients of AFRS is indistinguishable from the mucoid impactions of patients with ABPA. The adjacent sinus mucosa has a mixed cellular infiltrate of eosinophils, plasma cells, and lymphocytes.[7,92]However, the most important aspect in the concept of AFRS is the allergy to fungi. It is believed that fungal allergens elicit Type-I and possibly Type–III mediated mucosal inflammation in the absence of invasion in an atopic host.[13,93] The clinical examination should consider historical and physical stigma of atopy (hay fever, asthma, eczema, inhalant allergy), as well as nasal polyposis[6,2].

ii. EOSINOPHILIC FUNGAL RHINOSINUSITIS:

Contrary to the prevailing belief that fungi were responsible for CRS in only a selected group of patients with distinct pathophysiology, Ponikau et al in 1999 demonstrated the presence of fungi in nasal mucus from 96% of patients with CRS and found type I hypersensitivity to be present in < 25% of their study group. They detected fungi along with eosinophil and eosinophil degraded products in mucus.[5,96] They coined the term 'eosinophilic fungal rhinosinusitis' (EFRS)[14]. Similar results were observed by Braun et al [97]and Polzehl et al[98].

iii. EOSINOPHIL MUCIN RHINOSINUSITIS:

Ferguson et al described the presence of eosinophilic mucin without the presence of fungi in a proportion of rhinosinusitis patients and named this entity eosinophilic mucin rhinosinusitis (EMRS)[15] and suggested that EMRS is a systemic disease with dysregulation of immunological control. In the analysis of pathophysiology of eosinophil related FRS, it has been suggested that fungal elements trapped in the mucus in sinuses are the source of antigenic material that stimulates IgE, IgG and IgA production.[16]

Various authors propose fungal rhinosinusitis to be a continuous spectrum of disease starting from the noninvasive to the acute invasive varieties with considerable overlap and transition from one form to another in the same patient. Rowe-Jones and Moore-Gillon in 1994 proposed chronic destructive but noninvasive (semi invasive)form of fungal rhinosinusitis.[17] It is categorized by sinus expansion and bone erosion, but with no histologic evidence of tissue invasion. In this state, the pathogens lead to progressive chronic inflammation intermediate between allergic sinus fungal ball, and chronic invasive state.

Categories of fungal rhinosinusitis[6]

Category	***Host immune status***	***Role of fungus***	***Major fungus isolated***	***Course***
Invasive				
1.Granulomatous invasive	Immuno Competent	Pathogen	*A. Flavus*	Indolent, chronic
2.Chronic invasive	Often diabetes mellitus, steroid therapy	Pathogen	*A. fumigatus*	Chronic
3.Acute invasive (fulminant necrotizing)	Immuno Compromised	Pathogen	*Zygomycetes Aspergillus spp.*	*Acute*
Noninvasive				
1.Localized colonization (Saprobic infestation)	Immuno competent	Saprobe	*Aspergillus* spp.	May or may not progress to other forms especially sinus fungal ball
2 Fungal ball (Mycetoma/Aspergilloma)	Immuno competent	Saprobe	*Aspergillus* spp.	Chronic
3.Eosinophil related				
AFRS	Atopic	Allergen	Dematiaceous fungi,*A.flavus*	Chronic
EFRS	Majority atopic	Activation of eoinophil	Dematiaceous fungi	Chronic
EMRS	Asthma,aspirin sensitivity,IgG1 deficiency	Unknown	Not present	Chronic

AFRS = Allergic fungal rhinosinusitis; EFRS = Eosinophilic fungal rhinosinusitis; EMRS = Eosinophilic mucin rhinosinusitis

EPIDEMIOLOGY OF FUNGAL RHINOSINUSITIS:

HOST FACTORS*:*

Inhalation of ubiquitous fungi like *Aspergillus* and *Zygomycetes* is an innocuous phenomenon. However, in the immunodeficient host, these fungi may breach host defenses and propagate within and along the blood vessels and nerves, infecting sinonasal tissue and creating an acidotic area of tissue necrosis that is ideal for continued fungal proliferation.[6] Widespread use of steroid is also an important cause of increased incidence of the disease.[26,28,29] The steroid acts by two ways – suppressing normal inflammatory cell response and by inducing a diabetic stage. Other risk factors found to be associated with development of invasive fungal rhinosinusitis include long-term antibiotic usage, indwelling catheters, nasal intubations, metabolic abnormalities, prolonged hospitalization, and sinus disease For AFRS, atopy defines the condition and persons with type I hypersensitivity to fungi are exclusively affected by the disease[92,93]. AFRS is also found more in persons with simple asthma and aspirin sensitive asthma.[94] However, prior sinus surgery seems to be a more important risk factor for development of sinus fungal ball[95]. It has been speculated that sinus fungal ball may develop in any poorly ventilated sinus and that fungal exposure and poor sinus ventilation may be the only risk factors that are required.[12]In a case-control study, endodontic treatment on maxillary teeth was found to be a strong risk factor for fungal ball of the maxillary sinus.[20]

Agent Factors:

Zygomycetes are by far the commonest cause of acute invasive fungal rhinosinusitis. The predominant *Zygomycetes* causing such disease is *Rhizopus oryzae*.[28,32] The most common septate fungi causing acute invasive FRS in the immunocompromised patient are *Aspergillus fumigatus* and *Aspergillus flavus* .In contrast to foreign literature, in the Indian scenario *A. flavus* is isolated in more than 80% of cases of AFRS, both in southern and northern parts of the country[6,22]. In granulomatous invasive FRS *A. flavus* is the commonest pathogen isolated. In contrast *A. fumigatus* causes most cases of chronic invasive FRS. Only 30 to 50% of the cultures from fungal ball show the growth of the causative fungi, which are usually *Aspergillus fumigatus* or *Aspergillus flavus* and occasionally *P. boydii*[100].

PATHOGENESIS:

Inhalation of ubiquitous fungi like *Aspergillus* and *Zygomycetes* is an innocuous phenomenon. However, in the immunodeficient host, these fungi may breach host defenses and propagate within and along the blood vessels and nerves, infecting sinonasal tissue and creating an acidotic area of tissue necrosis that is ideal for continued fungal proliferation.[6] Widespread use of steroid is also an important cause of increased incidence of the disease.[26,28,29] The steroid acts by two ways – suppressing normal inflammatory cell response and by inducing a diabetic stage. Other risk factors found to be associated with

development of invasive fungal rhinosinusitis include long-term antibiotic usage, indwelling catheters, nasal intubations, metabolic abnormalities, prolonged hospitalization, and sinus disease. Conidia of aspergilla are often present in ambient air, but large amounts of them are present in dust, decomposing organic matter and soil. So, inhalation is the most common route of entry of the fungi into the sinus .The pathogenesis of mucormycosis is unclear and although the source is undoubtedly exogenous, possible sources have only been occasionally suggested. e.g adhesive dressings and air conditioning filtering units[25].

The fungi causing different categories of fungal rhinosinusitis are as follows. [6]

Category of fungal rhinosinusitis		Commonly isolated fungus	Rarely isolated fungus
Granulomatous invasive FRS		A. flavus	
Chronic invasive FRS		*A. fumigatus, less commonly A. flavus*	*Mucor, Alternaria alternata, Candida, Drechslera, Bipolaris hawaiiensis,Sporothrix schenckii, Pseudallescheria boydii,* *Exserohilum spp, Fusarium spp*
Localized colonization		*Aspergillus fumigatus, other Aspergillus spp.*	*Alternaria alternata, Penicillium rugulosum, mycelia sterilia, mucoraceous fungi.*
Fungal ball (Mycetoma/ Aspergilloma)		*Aspergillus fumigatus, Aspergillus flavus and occasionally P. boydii.*	*Chaetomium globosum, Scedosporium prolificans, Aspergillus nidulans,* *Penicillium spp.Schizophyllum commune, very rarely zygomycetes.*
Eosinophil related FRS	AFRS	*Dematiaceous fungi in USA* *Alternaria alternata, Bipolaris spp., Drechslera spp Curvularia lunata, Exserohilum.* *Aspergillus flavus in India and Middle East.*	*Schizophyllum commune,Aspergillus nidulans, Epicoccum nigrum,Penicillium sp. and Cladosporium spp.*
	EFRS	Similar to AFRS	

AFRS = Allergic fungal rhinosinusitis; EFRS = Eosinophilic fungal rhinosinusitis; EMRS = Eosinophilic mucin rhinosinusitis

PATHOGENESIS:

Signs and symptoms seen with fungal infections are as follows[21,22]

1. Nasal obstruction	2. Rhinorrhoea
3. Olfactory disturbance	4. Facial pain /headache
5. Facial fullness	6. Anosmia/ hyposmia
7. Proptosis	8. Visual impairment
9. Focal neurological deficits	10. Seizures
11. Altered sensorium	12. Purulence in nasal cavity
13. Halitosis	14. Fatigue/dental pain

INVESTIGATIONS:

A battery of investigations are done for all the cases of CFRS.they include[21]

- Total count, differential count,absolute eosinophil count for diagnosing allergic fungal sinusitis.
- Total serum IgE ,Blood sugar levels.
- Liver function tests,HIV testing .
- Anergy panel for cellular and humoral immunity.

PLAIN RADIOGRAPHY:

May show mucoperiosteal thickening with homogenous and complete sinus opacification. Radiologic evidence of sinusitis of one or more paranasal sinuses with or without flocculent calcifications is supportive of allergic FRS[21].

COMPUTED TOMOGRAPHY:

It is the imaging study of choice .It shows typically a rim of soft tissue attenuation along the bony walls of the involved sinus that is completely or almost completely opacified in fungal ball. Allergic fungal sinusitis may show bony erosion or deformity. A typical feature is the presence of hyperdensity amid soft tissue opacity of the sinus lumen.[6]Chronic invasive fungal disease typically demonstrates significant soft tissue thickening and evidence of altered adjacent bone[58].

MAGNETIC RESONANCE IMAGING:

MRI is recommended for impending invasion into the orbit and intracranial compartment. In AFS, MRI will typically reveal mild to moderate signal intensity on T1 weighted images with loss of signal intensity with T2 weighted images[21].

CHEST XRAY:

It should be considered in patients with allergic fungal sinusitis and pulmonary symptoms.

DIAGNOSTIC NASAL ENDOSCOPY:

Findings may include:[21]

- Fungal tufts –growing on retained secretions
- Polypoidal swellings /polyps
- Allergic mucin may be seen in cases of allergic fungal sinusitis.(golden yellow peanut butter like)
- Soft cheese like material(white to brown/black)
- Brown concretions ,Granulomatous mass
- White necrotic debris ;Black mucosal eschar

HISTOPATHOLOGY:

Histopathological appearance of lesions is an usual adjunct in establishing the diagnosis, prognosis and for deciding treatment protocols[55,8]

1) GRANULOMATOUS INVASIVE FRS:

Histopathologically, Noncaseating granuloma with foreign body or Langhans'type of giant cells may be seen, sometimes with vasculitis, vascular

proliferation and perivascular fibrosis. Hyphae in many occasions are scanty and are present inside the giant cells.[7,52]

2) CHRONIC INVASIVE FRS:

In contrast to Granulomatous invasive FRS, the entity is characterized as dense accumulation of hyphae, occasional presence of vascular invasion, sparse inflammatory reaction, and involvement of local structures.[7,52,87]

3) FUNGAL BALL:

The fungal ball is diagnosed microscopically by the marked absence of significant inflammatory cell infiltrate and abundance of tightly packed fungal hyphae. Surrrounding mucosa demonstrates chronic inflammatory infiltrate with mild to moderate plasma cell and lymphocyte infiltrate.[90,91]

4) ALLERGIC FUNGAL SINUSITIS:

The features are Scattered fungal hyphae in mucinous material with abundant eosinophils and Charcot leyden crystals. Allergic mucin is characterized by clumps of eosinophil and other cellular debris, within a background of pale eosinophilic -basophilic, amorphous mucin. The fungal elements tend to be sparse and are without subepithelial tissue invasion or fungal ball format[55].

SPECIMEN COLLECTION AND PROCESSING:

The collection, transport and processing of clinical specimens encompass one of the most important considerations in determining the etiology of fungal disease.

IDEAL SAMPLE:

Surgical samples should be transported in a sterile container .A few drops of sterile saline may be added to keep the sample moist. Fungal viability may be affected by excessive heat or cold. So room temperature transport and storage ideally within 2 hours is recommended.[23,3]

DIRECT EXAMINATION :

Hyphal elements and details of hyphal morphology of aspergilli can be readily observed in routine 10 % Potassium hydroxide preparations without or with a fluorescent compound such as Calcoflour white or in tissue sections by fungal stains such as Gomori methenamine silver staining[23].

As mucorales are common lab contaminants, microscopic demonstration of the presence of mucorales in clinical material taken from necrotic lesions is more significant than isolation of the same in culture. In contrast to *aspergillus, Zygomycetes* are larger, do not have parallel walls with 45 ^{0} angle branching, and do not radiate from a single point in tissue. Furthermore, mucorales stain

poorly by Periodic acid-schiff stain but stain well with H & E stain and Gomori stain. Another stain that is useful is Cresyl Fast Violet which stains the Zygomycete wall brick red and other fungi blue or purple.[23]

ANTIGEN DETECTION:

In patients with invasive disease, antigen detection may be very useful. Several tests for detection of soluble antigens of *Aspergillus* spp in serum, urine or other body fluids have been developed. Radio immunoassay, Enzyme linked immune sorbent assay,Biotin avidin linked immunosorbent assay, Latex agglutination and Immunoblotting have been the most commonly used method, but only a few of them are commercially available.e.g a latex agglutination test, Pastorex *Aspergillus* and a ELISA Platelia *Aspergillus* are available. Regardless of the test used ,success of detecting antigenemia is directly related to the frequency of monitoring of samples,The Platelia ELISA detected antigen before diagnosis was made by other means in approximately two third of the patients [23,1].These tests identified the *Aspergillus* Galactomannan.

Limitations:[23]

- Use of antifungals significantly reduces the sensitivity of the assay.
- ELISA reactivity noted with treatment with β lactam antibiotics(may be because *Penicillium species* are used for drug production)

- False positive with beta glucan occur in patients with renal failure undergoing dialysis with cellulose membranes and those treated on immunoglobulin products.

There are no routinely available antigen detection formats for the diagnosis of Mucormycosis and agents of other hyalohyphomycosis. Tests that detect(1-3)β –D-Glucan,a characteristic cell wall component of a broad range of fungal pathogens has also been developed but clinical experience is limited. β –D-Glucan is detected by a glucan assay on the basis of its recognition by the immune system of Horseshoe crab ,specifically *Tachypleus tridentatus* and *Limulus polyphemus* . Factor G is activated by the glucan .The Limulus lysate assay and BG specific variant Fungitell assay has been approved for use.This assay is manufactured by removing bacterial endotoxin sensitive factor C from limulus lysate making this reagent specific for beta glucan. This modified lysate is formulated with a synthetic chromogenic substrate and salts. The sensitivity and specificity are 69.9% and 87.1% respectively.[24]

NUCLEIC ACID DETECTION TECHN IQUES:

Though highly sensitive and specific , they are still in experimental stage. Different approaches have been tried to detect a broad range of fungi in the first step and to identify to species level in the second step. Further, technical advances in post amplification analysis have enabled real time detection and quantification of fungal DNA load in tissue or blood samples.[1]

TYPING SYSTEMS:

It is done mainly for epidemiological studies. Restriction fragment length polymorphism analysis, Random amplified polymorphic DNA analysis and repetitive element and /or complex probes with southern blotting are the methods available. Genotyping has suggested that aspergillosis has a nosocomial origin in some cases[23].

ANTIFUNGAL SUSCEPTIBILITY TESTING: CLSI M 38 A DOCUMENT FOR MICROBROTH DILUTION OF FILAMENTOUS FUNGI:[34]

This document is the reference method of susceptibility testing of filamentous fungi. This provides a standard basis from which other methods can be developed.[34]

Suitable for	Conidium-and spore forming fungi
Inoculum	$0.4x10^4$-$5x10^4$ CFU/ml
Inoculum Standardization	Spectrophotometrically
Test medium	RPMI 1640
Format	Microdilution
Temperature	35°C
Duration of incubation	24 h/48h
Endpoint	No visible growth

AGAR DILUTION:

Agar dilution method has been done in yeast nitrogen base agar with good reproducibility[51]

E TEST:

Etest is a commercially available method and directly quantifies antifungal susceptibility in terms of discrete MIC values. For *Aspergillus* spp., good correlations with Amphotericin B and Itraconazole Etest and M38-A method have been demonstrated. Etest was superior in detecting caspofungin resistance in *A. fumigatus*, when compared with EUCAST and CLSI methodology. [37,39]

DISK DIFFUSION:

Disk diffusion interpretive criteria are available by the latest CLSI document.Espinel –Ingroff et al in a multicenteric evaluation, have studied the disk diffusion assay for filamentous fungi[45] and concluded that the optimal conditions were (i)plain Mueller Hinton agar,(ii) incubation times of 16-24 hours for zygomycetes, 24 hours for *Aspergillus fumigatus,A.flavus, A.niger* and 48 hours for other species and (iii) Itraconazole, Amphotericin10µg, Posaconazole 5µg, Voriconazole 1 µg, Caspofungin 5 µg disks.

Sensititre® YeastOne™ Test Panel . This is a microtitre broth dilution method based on the CLSI M27-A2 standard described above. Each test consists of a disposable microtitre plate, which contains dried serial dilutions of six antifungal agents, Amphotericin B (range 0.008-16 µg/ml), Fluconazole (range 0.125-256 µg/ml), Itraconazole (range 0.008-16µ g/ml), Ketoconazole (range 0.008-16 µg/ml) and 5-Flucytosine (range 0.03-64 µg/ml), Voriconazole (range 0.008-16 µg/ml) in individual wells . The wells also contain Alamar Blue as a colorimetric indicator, which greatly improves the end point readability by a colour change from blue to pink. Results are expressed as an MIC and comparative studies against the CLSI method have shown favorable results [48]. Excellent shelf life and the test also works with moulds, especially those that sporulate freely like *Aspergillus*[40,41].

Neo-Sens*it*ab:

This is a simple agar diffusion method using tablets to determine the susceptibility of fungi to antifungal agents. Once again there have been problems with which media to use and with the interpretation of the end points. Recent studies have used Mueller-Hinton agar supplemented with 2% glucose and 0.5 mcg/ml methylene blue as the medium[49] (CLSI M44-P method) and a Biomic plate reader to electronically read and interpret zones sizes. However, individual disk zone sizes are often not able to differentiate between Susceptible and Susceptible Dose Dependent isolates and the correlation

between zone size and MIC is more variable[48]. Once again, resistant isolates need to be confirmed by using one of the appropriate CLSI methods.

TREATMENT:

NON INVASIVE FUNGAL SINUSITIS:

1.SUPERFICIAL MYCOSIS/FUNGAL BALL:

Treatment includes mainly debridement of involved sinus. Antifungal agents are not used. Culture directed antibiotics to combat co existent bacterial infection may be used.

2. ALLERGIC FUNGAL SINUSITIS:

Allergic fungal sinusitis is best managed with an aggressive combination of medical and surgical therapy. Complete surgical drainage with restoration of sinus aeration and mucociliary clearance is a corner stone of therapy, but it is alone insufficient to manage the condition. Medical management includes culture directed antibiotics, mucolytic therapy, antihistamines, systemic steroids, immunotherapy and /or anti fungal chemotherapy. Itraconazole has been used for allergic fungal sinusitis in conjunction with an initial burst of systemic steroids.[21]

INVASIVE FUNGAL SINUSITIS:

1. CHRONIC INVASIVE FUNGAL SINUSITIS:

This condition is best handled by a combination of medical and surgical treatments. Wide local resection is preferred in combination with appropriate antifungal therapy. [21]

2. ACUTE INVASIVE FUNGAL SINUSITIS:

Debridement of all grossly infected and devitalized tissue is mandatory. Orbital exenteration in patients with known cerebral involvement and very poor vision may help reduce the burden of infected tissue. Wound packing that is impregnated with Amphotericin can be used. Following surgery, irrigation of nasal cavity with Amphotericin B(50 mg /L of water)irrigations(20 ml 4 times a day) may be performed. Other therapies that have been tried include hyperbaric oxygen and G-colony stimulating factor infusion. Despite aggressive therapy and surgical debridement, the mortality rate is very high. Overall survival in diabetic patients approaches 80 % when underlying ketosis is corrected.[21]

ANTIFUNGAL THERAPY:

Therapy is indicated only for mold infections of the sinuses .Candida spp are not implicated as a cause of fungal sinusitis though asymptomatic

colonization of sinuses are often present ,hence antifungal chemotherapy is not usually advocated against them.

Clinically useful antifungals available for moulds:

- Polyenes: Amphotericin B,Amphotericin B lipid formulation
- Azoles: Itraconazole,Voriconazole,Posaconazole
- Echinocandins: Caspofungin,Micafungin,Anidulafungin

AMPHOTERICIN B:

Mechanism of action: It binds to ergosterol in fungal cytoplasmic membrane, increasing permeability and causing leakage of intracellular components. Membrane channel activity is increased at lower doses and pores are formed at higher doses[52]

Spectrum of activity: Good activity against most *Candida* species, *Aspergillus spp, Cryptococcus spp.* and dimorphic moulds. Dosage:0.7 to 1.5 mg/kg/day

LIPID FORMULATIONS OF AMPHOTERICIN B:

Three formulations available:

- ✓ Amphotericin B colloidal dispersion
- ✓ Amphotericin B lipid complex
- ✓ Liposomal amphotericin B

FDA indications:

- Fungal infections intolerant/refractory to amphotericin
- Empirical therapy in febrile neutropenics

Dosage: 3-6 mg/kg/dose iv.

AZOLES[52,24]:

ORGANISM	ITRACONAZOLE	VORICONAZOLE	POSACONAZOLE
A.fumigatus	+	++	++
A.flavus	++	++	++
A.terreus	++	+	++
Fusarium	-	-/+	-/+
Rhizopus spp	-/+	-	+
Mucor spp	-/+	-	-
Scedosporium apiospermum	+	+/++	+/++
S.prolificans	-	-/+	-
Dematiaceous fungi	+/++	+/++	+/++

Mechanism of action:

Inhibition of cytochrome P-450- dependent lanosterol 14-demethylase, an enzyme required for the synthesis of ergosterol, the main component of fungal cell membranes. This results in the accumulation of methylated sterols , depletion of ergosterol and inhibition of cell growth.[52]

Dosage:

Itraconazole: 200 mg b.i.d

Voriconazole 6 mg/kg q12 h IV OR 200 mg q12 h

Posaconazole 100 mg b.i.d

Indications:

Itraconazole: Invasive aspergillosis refractory to amphotericin.
Voriconazole: Approved as primary therapy in invasive aspergillosis.

Posaconazole: Prophylaxis of invasive fungal infections.

Shown to have good activity against zygomycetes.

ECHINOCANDINS:[24]

MECHANISM OF ACTION:

Mechanism of action is noncompetitive inhibition of enzyme glucan synthase which produces (1,3)β d glucan. The destruction of cell wall structure leads to osmotic instability and ultimately lysis of the fungal cell[52].

Caspofungin : 70 mg iv loading dose followed by a daily 50 mg IV dose[52].

INDICATIONS:

It is indicated in the treatment of invasive aspergillosis in patients who are refractory to or intolerant of other antifungals.It is also approved as empirical therapy for presumed fungal infections in neutropenic patients.

MATERIALS AND METHODS

PLACE OF STUDY:

This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College in association with Upgraded Institute of Otorhinolaryngology, Rajiv Gandhi Government General Hospital, Chennai. All patients undergoing functional endoscopic sinus surgery (FESS) and/or diagnostic nasal endoscopy (DNE) were both taken under the study.

STUDY PERIOD:

The study period was from January 2010 to June 2011.

ETHICAL CONSIDERATION:

Approval was obtained from the Institutional Ethical Committee before the commencement of the study. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire.

STUDY POPULATION:

All consecutive Patients >18 years of age within the study period with

- Radiologically proven sinusitis with
- Symptoms > 12 weeks duration

- whose DNE/ FESS sampling or clinical condition is suggestive of fungal involvement in the pathogenesis of the disease were included in the study.

EXCLUSION CRITERIA:

Patients with symptoms of sinusitis < 12 weeks duration and age<18 years were excluded from the study.

DATA COLLECTION :

Data collection included name, age, sex, address, date of admission, diagnosis at admission, physical examination findings and Demographic profile which include H/O asthma, aspirin allergy, peripheral blood eosinophilia, Diabetes mellitus, Chronic eczema/dermatitis, COPD, Uremia/chronic kidney disease, neoplasm, immunosuppressive therapy, recurrence and injury /trauma to the sinuses.

STATISTICAL ANALYSIS:

Statistical analysis were carried out using Statistical Package for Social Sciences (SPSS) and Epi-Info softwares by a statistician. The proportional data of this cross sectional study were tested using **Pearson's Chi Square analysis test** and **Fisher exact probability test** .

CASE DEFINITIONS:

INVASIVE FUNGAL INFECTIONS:

Diagnostic criteria for invasive fungal infections as defined by deShazo:[9]

- Mucosal thickening or air fluid level compatible with sinusitis on radiography.
- Histopathological evidence of hyphal forms within the sinus mucosa , submucosa,blood vessel or bone.

To diagnose **GRANULOMATOUS INVASIVE SINUSITIS**, histopathological evidence of hyphal forms within the sinus mucosa ,submucosa, bloodvessel or bone in association with granuloma containing giant cells.

FUNGAL BALL:

Diagnostic criteria for fungal ball as defined by deShazo[12]

- Radiological studies showing sinus opacification often associated with floccular calcifications.
- Mucopurulent cheesy clay like material presenting at a single sinus at time.
- Histopathological evaluation showing dense agglomeration of hyphae separate from adjacent respiratory mucosa and absence of allergic mucin.
- No fungal invasion of tissue or mucosa.

ALLERGIC FUNGAL RHINOSINUSITIS(AFRS):

Patients with a combination of the following findings were diagnosed as having. AFRS as per diagnostic criteria described by Bent and Kuhn:[4]

- Radiologically proven sinusitis.
- Presence of allergic mucin within nasal cavity or sinuses.
- Demonstration of fungal hyphae in allergic mucin.
- Absence of fungal invasion in histopathology
- Absence of diabetes, immunodeficiency or recent treatment with Immunosuppressants

Invasive fungal infections are defined in terms of "PROVEN", "POSSIBLE"; "PROBABLE"[35].

PROVEN:

- POSITIVE culture obtained by a sterile procedure from a normally sterile site and clinically and radiologically abnormal site consistent with infection

PROBABLE:

- Atleast one criteria from host section ,one microbiological criteria and one major or two minor clinical criteria from an abnormal site consistent with infection.

POSSIBLE:

- Atleast one criteria from host section and one microbiological or one major (or two minor) clinical criteria from an abnormal site consistent with infection.

CRITERIA:

Host factors:

- Neutropenia(>500/mm^3 for >10 days) or coexistent AIDS.
- Persistent fever >96 hours refractory to antibiotics
- Body temperature> 38^oC or <36^oC
- Recent or current use of immunosuppressive agents or steroids>3 weeks

Microbiological criteria:

- Positive result of culture or findings of cytological /direct microscopic evaluation for mould from sinus aspirate sample

Major:

- Suggestive radiological evidence of invasive infection in sinuses(involvement of sinus walls, neighbouring structures and skull base)

Minor:

- Upper respiratory tract infections
- Nose ulceration or eschar
- Periorbital swelling
- Maxillary tenderness
- Perforation of hard palate

SAMPLE COLLECTION:

Sample collection was done according to American Thoracic Society Recommendations for collection of specimen for fungal culture.[31]

Tissue biopsies or endoscopic aspirates were transported immediately in a sterile gauze moistened with physiologic, sterile saline solution in a screw capped sterile container. Care was taken so that the specimen was not frozen or allowed to dehydrate before culture.

CRITERIA FOR REJECTION:

- Improperly labelled samples
- Samples that are transported in unsterile containers
- Samples that have leaked or show signs of dehydration
- Samples received in formalin[31]

As the sample is collected, the endoscopic grading of AFS[46] as described by Kupferberg et al is also noted if applicable(for AFRS):

GRADE 0: No evidence of disease

GRADE 1: Edematous mucosa+allergic mucin

GRADE 2: Polyps+allergic mucin

GRADE 3: Polyps and fungal debris

PROCESSING OF SPECIMENS:

When processing tissue for the recovery of fungi, the use of a mortar or tissue grinder was avoided[31,33]. The tissue was minced into 1 mm cubes with a sterile scissors or a sharp scalpel blade and the tiny fragments were placed directly on the agar. Sabouraud Dextrose Agar was used for primary isolation.[31]

DIRECT EXAMINATION:

POTASSIUM HYDROXIDE (KOH)MOUNT PREPARATION:[31,33]

A clean, grease free glass slide was taken. One large drop of 10% KOH solution was placed on the slide. A small quantity of the specimen was mixed in the KOH drop. A clean coverslip was placed over the drop. The slide was placed in a moist chamber at room temperature. Tissue usually takes 20-30 minutes to clear. It is observed under low and high power for the presence of yeasts or hyphal forms. Simultaneously the specimen was also processed in

Institute of Pathology, Rajiv Gandhi Government General hospital. Haematoxylin and Eosin stain was done routinely. Special stains like Giemsa, Periodic acid Schiff and Gomori Methenamine silver staining were also done in case of suspicious fungal forms in H &E stain.

CULTURE:

A minimum of 5 ml[31] of tissue homogenate was inoculated onto 2 slants of Sabouraud Dextrose Agar with antibiotics Gentamicin added at a concentration of 20mg/litre[32]. Inoculated tubes were incubated at 25 and 37 ^{O} C. Cultures were examined for expected growth, daily in the first week and twice a week for the subsequent period. Cultures were incubated for a minimum of 4 weeks and in some cases upto six weeks before being discarded as sterile.[32]

INTERPRETATION OF FUNGAL CULTURES:

The following features were considered before labelling an opportunistic fungi that are otherwise considered as contaminants as pathogen:[32]

- Isolation of same strain in all culture tubes
- Repeated isolation of same strain in multiple specimens
- Immune status of the patient
- Direct microscopic detection of fungal forms

DENTIFICATION OF FUNGAL ISOLATES:

All isolates were systematically identified by standard techniques.

Various mounting methods done include

1) Tease mount
2) Scotch tape
3) Slide culture technique

1) Tease mount:

A small drop of lactophenol cotton blue (LPCB) was placed on a clean microscopic slide. A small portion of growth was removed midway between the colony center and edge. The removed colony was placed on a drop of lactophenol cotton blue on the slide. The growth was teased using a pair of dissecting needles so as to have a thin spread out. The coverslip is placed gently at the edge of the drop of mounting fluid avoiding trapping of air bubbles.[32,33]

2) Scotch tape technique:

A drop of mounting fluid was placed on the slide.A 2 cm long tape was taken and one end was touched to a forceps /stick and the other end to the colony. The tape with the surface containing fungus was laid face down into

the mounting medium on the slide.The tape was detached from the stick and mount examined [32]

3) Slide culture technique: Setup: In a 100 mm glass petri dish, a filter paper, V-shaped glass rod, a slide and a coverslip placed . The whole setup is autoclaved at 121^0C for 15 minutes at 15 pounds[33.]

Procedure:

1 cm square agar was cut aseptically from potato dextrose agar. The agar block was transferred to the slide in the setup. A very small amount of the colony was transferred to the four sides of the agar block. A coverslip was placed on the inoculated agar block.1-1.5 ml of sterile water was added to the filter paper. 5%glycerin was added to the sterile water to prevent condensation of moisture on the slide. Slide culture was incubated in the dark at room temperature till good sporulation occurs.

Removing the slide culture:

A small drop of mounting fluid was placed on a slide.With forceps,the cover slip was carefully removed.A drop of 95 % alcohol was added to the cover slip to wet the colony and to prevent trapping of air bubble. The cover slip was placed carefully on the mounting medium. The excess of mounting fluid was removed and the mount was examined under microscope.[33]

MOULD IDENTIFICATION SCHEME [33]

This includes

- Growth rate
- Colony characteristics;
    - Texture ,Colour(obverse ,reverse)
- Microscopy:
    i. Fruiting structures: Synnemata, Pycnidia,
    Ascocarps(Gymnothecia,Cleistothecia,Perithecia)
    ii. Hyphae: Colour, Size, Septation, Special Structures
    iii. Conidiogenesis: Conidiogenous cell,Proliferation of conidiophore
    .

ANTIFUNGAL SUSCEPTIBILTY TESTING:[33,32,31,34]

Amphotericin B and Itraconazole powders were obtained from HiMedia, Mumbai and Pharma Fabricon respectively. Their assay potency were 750 µg/mg each.

$$\text{Weight (mg)} = \frac{\text{volume (ml)} \times \text{desired concentration (µg/ml)}}{\text{Assay potency (µg/ml)}}$$

$$\text{Volume(ml)} = \frac{\text{weight (mg)} \times \text{assay potency(µg/ml)}}{\text{concentration (µg/ml)}}$$

STOCK SOLUTION:

Solvent used is Dimethyl sulfoxide(DMSO) for Amphotericin B and Itraconazole. Stock solution of 1600 µg/ml is prepared. A series of dilutions at 100 times the final concentration was prepared from the antifungal stock solution in the same solvent. Each intermediate solution was then further diluted to final strength in the test medium. This procedure was done to avoid dilution artifacts that result from precipitation of compounds with low solubility in aqueous media.

Media : RPMI 1640(with glutamine, without bicarbonate,and phenol red as pH indicator), HiMedia, Mumbai.

Inoculum preparation:

All organisms were subcultured onto Potato dextrose agar , incubated at 35^{o}C for 7 days. The culture was covered with 1 ml of sterile 0.85% saline and a suspension prepared by gently probing the colonies. Addition of 1 drop of Tween 20 will help dispersion of Aspergillus conidia.The resulting mixture of conidia and hyphal elements was withdrawn and transferred to a sterile tube and allowed to settle. The uniform suspension was transferred to a screw capped tube and vortexed. The densities of the conidia or the sporangiospore suspensions were read and adjusted to a optical density of 0.09-0.11 for A*spergillus* spp and 0.15-0.17 for Rhizopus spp by spectrophotometry. These

will be diluted 1:50 in the standard medium. This will give a density needed of approximately $0.4x10^4$ to $5x10^4$ CFU/ml when mixed with the antifungal agent.

INCUBATION: All microtitre plates were incubated at 35°C. Examination time for Rhizopus: 21-26 hours of incubation and Aspergillus spp: 46-50 hours of incubation.

INTERPRETATION:

Minimum inhibitory concentration is the lowest concentration of an antifungal that substantially inhibits growth of the microorganism as detected visually. Each microdilution well was then given a numerical score as follows;

Score 4 - No reduction of growth

Score 3 - Slight reduction in growth(75 % of growth control)

Score 2 - Prominent reduction in growth(50 % of growth control)

Score 1 - Optically clear or absence of growth

One growth control well and one antifungal control well were also set up. Recommended MIC limits of reference strain *ATCC A.flavus* 204304 which was also put up as quality control.Amphotericin B : 0.5-4 µg/ml, Itraconazole: 0.2-0.5µg/ml.

PROCEDURE:

Starting conc	1600	2	4	8	16	32	64	128	256	512	Remarks
Tube(T)	T1stock	2x	4x	8x	2x	4x	8x	2x	4x	8x	
		T2	T3	T4	T 5	T6	T7	T8	T9	T10	
Add drug(ml) +	From T1 -	From T1 0.5 +	From T1 0.5 +	From T1 0.5 +	From T4 0.5 +	From T4 0.5 +	From T4 0.5 +	From T7 0.5 +	From T7 0.5 +	From T7 0.5 +	Step1
Add DMSO (ml)	-	0.5	1.5	3.5	0.5	1.5	3.5	0.5	1.5	3.5	Row 1
Intermediate drug conc.	1600	800	400	200	100	50	25	12.5	6.25	3.313	
Add drug from row 1above	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	0.1 +	Step 2 Row 2 (1:50)
RPMI (ml)	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	
Final conc at 1:50(µg/ml)	32	16	8	4	2	1	0.5	0.25	0.125	0.0625	(2x)
From row 2 add drug to microtitre	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	Step 3(1:1)
Add inoculum to plate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	Step 4
Final drug conc. In well (µg/ml)	16	8	4	2	1	0.5	0.25	0.125	0.0625	0.0313	

CLINICAL SIGNIFICANCE:

AMPHOTERICIN: MIC above 2 µg/ml have been associated with treatment failure and MIC below 2 µg/ml with clinical cure.

ITRACONAZOLE: Preliminary data indicate that high Itraconazole MICs (MICs>8 µg/ml)are associated with clinical resistance to the drug.Data are not

available to indicate a correlation between MIC and outcome of treatment with Itraconazole.

DISC DIFFUSION METHOD AND E TEST:

Inoculum transmittance was adjusted according to CLSI M38-A protocol as described above for microbroth dilution. Suspensions were applied to the surface of the agar media by using swab applicators;Mueller Hinton agar for disk diffusion[45] and RPMI agar for E test [44]The inoculated plate was allowed to dry for 15 minutes .Amphotericin B 10µg and Itraconazole 10µg disks were applied on Mueller Hinton agar.[44]Estrip for Amphotericin B was applied onto the inoculated RPMI agar[43]**.**Zone diameters were measured in the disk diffusion assay to the nearest whole millimeter at the point where there was a prominent reduction of growth after 16-24 hours for zygomycetes and after 24,48 and 72 hours for the other species. E test was read after 24 hours or when there was sufficient growth to take a reading.[43,36,37,38.]Zone diameter categories were[44]

DRUGS	SUSCEPTIBLE	SUSCEPTIBLE DOSE DEPENDENT	RESISTANT
AMPHOTERICIN B	≥15 mm	13-14 mm	≤12 mm
ITRACONAZOLE	≥17 mm	14-16 mm	≤13 mm

AGAR DILUTION:

Stock solutions and Drug dilutions were prepared according to the CLSI M 38 A guidelines. For susceptibility testing, 1ml of Yeast nitrogen base was thoroughly mixed with 18ml of molten agar (Himedia, Mumbai) and 1 ml of corresponding drug dilution and poured in 100mm sterile petri plates. Plates were dried prior to use. The inoculum concentration was adjusted to 1.0 $X10^6$ cells per ml establishing 90% transmission at 530 nm by the method of Shadomy and Espinel-Ingroff [50]. A 0.01-ml amount (1.0 x 10^4 spores) was delivered onto the surface of agar media in 100-cm^2 petri dishes. A control SDA plate (20 ml of SDA) and a plate for each concentration of 0.125 to 16 μg/ml serial dilutions were inoculated. Plates were incubated at 30°C for 48 h. The MIC was defined as the lowest concentration which caused greater than 80% inhibition of growth compared with the growth on the control plate. This definition, rather than a 100% inhibition endpoint, eliminated films and proved reproducible results in preliminary experiments.

RESULTS

This study was conducted among a total of 380 cases of Chronic Rhinosinusitis who underwent Functional endoscopic sinus surgery and Diagnostic nasal endoscopy at the Upgraded Institute of Otorhinolaryngology during the study period. 80 cases which fulfilled the inclusion criteria were included in the study. Of the 80 cases, 43 cases were recognized as chronic fungal Rhinosinusitis .Overall incidence of FRS was 11.3% in this study.

TABLE 1

AGE AND SEX DISTRIBUTION OF PATIENTS WITH CFRS AND NFRS.

Age distribution		CFRS(n=43)								NON CFRS (n=37)	
		AFRS(n=29)		FB(n=2)		CGFRS(n=4)		CIFRS(n=8)			
		M	F	M	F	M	F	M	F	M	F
Young adults	21-30	7 (24%)	6 (21%)	-	-	-	-	1 (12%)	1 (12.5%)	4 (11%)	1 (3%)
	31-40	- (0%)	5 (17%)	-	-	1 (25%)	-	- (0%)	- (0%)	1 (3%)	5 (14%)
	Total	**7 (24%)**	**11 (38%)**	-	-	1 (25%)	1 (25%)	1 (12.5%)	1 (12.5%)	5 (14%)	6 (17%)
Middle age	41-50	2 (7%)	2 (7%)	-	2 (100%)	1 (25%)	-	2 (25%)	1 (12.5%)	7 (19%)	7 (19%)
	51-60	2 (7%)	3 (10%)	-	-	1 (25%)	-	1 (12.5%)	1 (12.5%)	3 (8%)	3 (8%)
	Total	4 (14%)	5 (17%)	-	2 (100%)	2 (50%)	-	**3 (38%)**	**2 (25%)**	10 (27%)	10 (27%)
Old age	61-70	-	2 (7%)	-	-	-	-	-	-	4 (11%)	1 (3%)
	71-80	-	- (0%)	-	-	-	-	1 (12.5%)	-	- (0%)	1 (3%)
	>80	-	-	-	-	-	-	-	-	-	-
	Total	-	2 (7%)	-	-	-	-	1 (12.5%)	-	4 (11%)	2 (5%)

P =0.038

Of the total 43 cases of fungal sinusitis,22 (51%)were in the age group of 21-40(young adults). An almost equal number 18 patients (41.8%) were in the age group of 41-60(middle age) and a minor number (3) of patients in the age group>60(6.9%). In statistical analysis, **p<0.05** was obtained. This is statistically significant. So, AFS predominated in the 21-40 age group (62%) whereas CIFRS predominated in 41-60 age group. P value for male /female association was **0.555** which is statistically insignificant. Therefore, gender does not make significant difference.

TABLE 2

COMPARISON OF PRE OPERATIVE SYMPTOMS IN PATIENTS WITH CFRS AND NON CFRS

Symptoms	**NFRS(n=37)**		**CFRS(n=43)**	
Nasal block	26	70 %	39	**90.6%**
Nasal discharge	33	89%	33	76.7%
Headache	36	**97%**	30	69.7%
Facial puffiness	-	-	3	6.9%
Proptosis	-	-	2	4.6%
Anosmia/hyposmia	24	65%	27	62.7%
Tinnitus	-	-	1	2.3%
RRTI #	24	65%	36	83.7%
Others*	-	-	5	11.6%

#Recurrent respiratory tract infections (**p=0.22**)

*Includes Diminished vision, altered sensorium, speech disturbances, nerve palsies.

Majority of patients of FRS presented with nasal block(90.6%) as the predominant complaint ,though Headache predominated as the presenting complaint(97%) in sinusitis of non fungal etiology,. Headache was the presenting complaint in only 69.7 % of the people with fungal sinusitis. Symptoms like proptosis, facial puffiness, tinnitus were more common in invasive fungal infections than in others. But this difference was not statistically significant.

TABLE 3
COMPARISON BETWEEN SITE OF INVOLVEMENT BETWEEN CFRS AND NON CFRS

SITE INVOLVED			CFRS n (%) (n=43)	Non CFRS (%) (n=37)
PANSINUSITIS			25 (58%)	31 (84%)
Maxillary Sinus	Bilateral		6 (14%)	3 (8%)
	Unilateral	R	2 (5%)	3 (8%)
		L	4 (9%)	- (0%)
Frontal Sinus	Bilateral		- (0%)	- (0%)
	Unilateral	R	- (0%)	- (0%)
		L	- (0%)	- (0%)
Ethmoid Sinus	Bilateral		3 7%)	- (0%)
	Unilateral	R	- (0%)	- (0%)
		L	- (0%)	- (0%)
Sphenoid Sinus	Bilateral		3 (7%)	- (0%)
	Unilateral	R	- (0%)	- (0%)
		L	- (0%)	- (0%)
Orbit Involvement*			4 (9.3%)	- (0%)

*observed along with other sinus involvement . **P=0.013**

Both Fungal and non fungal sinusitis involved all the sinuses in 58% and 84% of cases .The next predominant was maxillary sinusitis that involved 24% of cases of CFRS and 16% of non CFRS. In statistical analysis, $p<0.05$ was obtained. So, it is a statistically significant fact that NFRS commonly presented as pansinusitis whereas FRS can affect even a single sinus.

TABLE 4

CATEGORIZATION OF THE CASES OF FUNGAL SINUSITIS n=43

CATEGORIES		MALE	FEMALE	TOTAL
ALLERGIC FUNGAL RHINOSINUSITIS		11 (**26%**)	18 (**42%**)	29 (**67%**)
FUNGAL BALL		- (0%)	2 (5%)	2 (5%)
CHRONIC FUNGAL GRANULOMATOUS SINUSITIS		3 (7%)	1 (2.3%)	4 (9%)
CHRONIC INVASIVE FUNGAL SINUSITIS	PROVEN	4 (9%)	2 (5%)	6 (14%)
	PROBABLE	1 (2.3%)	1 (2.3%)	2 (5%)
	POSSIBLE	-	-	-

Allergic fungal sinusitis was the most common form of sinusitis that was noted. This contributed to 67 % of the cases. This was followed by 19 % of chronic invasive fungal sinusitis .There was no statistically difference in sex distribution of the cases.

TABLE 5

COMPARISON BETWEEN RISK FACTORS ASSOCIATED WITH CHRONIC INVASIVE FUNGAL SINUSITIS AND OTHER CATEGORIES OF CFRS.

Risk Factors	No. Of CIFRS (n=8)	OTHER CATEGORIES (AFRS, CGFRS, FB) (n=35)			
		AFRS (n=29)	CGFRS (n=4)	FB (n=2)	Total
Hyperglycemia	**6 (75%)**	2 (6.8%)	-	-	2 (6%)
Chronic Kidney Disease	1 (12.5%)	1 (0.2%)	-	-	1 (0.2%)
Renal Transplant	1 (12.5%)	-	-	-	- (0%)
Asthma/Chronic eczema	-	**16 (55%)**	-	-	16 (46%)
None	1 (12.5%)	17 (58.6%)	4 (100%)	2 (100 %)	23 (66%)

BY CHI SQUARE TEST: **(P=0.002)**

In statistical analysis, $p<0.05$ was obtained. So, it is a statistically significant fact that Hyperglycemia was a significant risk factor that favoured invasive forms of fungal sinusitis. Asthma was also a significant risk factor known in cases of AFRS. Comparison between presence of asthma in AFRS and other categories showed a P value of 0.002 which was statistically significant.Few patients had multiple risks.(diabetes+CKD,diabetes+asthma).

TABLE 6

CORRELATION BETWEEN ENDOSCOPIC STAGING [45] AND RECURRENCE RATE OF ALLERGIC FUNGAL RHINO SINUSITIS, n=29

Stage	Description	Number observed	Percentage	Recurrence	%	
0	No evidence of disease	-	-	-	0%	
I	Edematous mucosa +allergic mucin		-	-	0%	
II	Polyps +allergic mucin	10	34.4%	4	13.7%	**P= 0.599**
III	Polyps +fungal debris	19	**65.5%**	11	**37.9%**	

A majority constituting 65.5 % of the allergic fungal sinusitis cases presented in a fairly advanced stage of the disease, i.e, stage III.61 % of recurrence was noted in stage III of the disease. The P value for the association between recurrence and stage of disease was 0.599.This association is statistically insignificant.

TABLE 7

COMPARISON BETWEEN DIRECT MICROSCOPIC OBSERVATION, HPE AND CULTURE EXAMINATION.

Total number of cases		**43**	**Sensitivity (%)**
Positive by	Direct examination(10% KOH mount)	41	**95.34**
	Histopathology	40	**93**
	Culture	38	**88.37**

Direct microscopy was able to clinch the diagnosis in 95.34 % of the cases, whereas Histopathology and Culture could do so in only 93% and 88.3% respectively.

TABLE 8

ETIOLOGICAL FUNGAL AGENTS OF CHRONIC FUNGAL SINUSITIS AND THEIR RELATIVE FREQUENCY OF ISOLATION

Species	**AFS (n=29)**	**Fungal Ball (n=2)**	**CGFRS (n=4)**	**CIFRS (n=8)**	**Total (n=43)**
A.flavus	16 (**55.1%)**	2 (**100%**)	1 (25%)	1 (12.5%)	20 (**46.5%)**
Rhizopus spp	1 (3.4%)	-	1 (25%)	4 (**50%)**	6 (13.9%)
A.fumigatus	3 (10.3%)	-	1(25%)	1 (12.5%)	5 (11.6%)
A.niger	3 (10.3%)	-	-	-	3 (6.9%)
A.nidulans	-	-	-	1 (12.5%)	1 (2.3%)
A.clavatus	1 (3.4%)	-	-	-	1 (2.3%)
A.versicolor	-	-	1 (25%)	-	1 (2.3%)
Penicillium spp.	1 (3.4%)	-	-	-	1 (2.3%)
Paecilomyces variotii	1 (3.4%)	-	-	-	1 (2.3%)
KOH +/NG	4 (13.8%)	-	-	2 (25%)	6 (13.9%)
Mixed growth	1 (3.4%)*	-	-	1 (12.5%)#	2 (4.6%)

#A.fumigatus+Rhizopus spp **A.niger+Rhizopus spp*

Majority of the fungi isolated were *Aspergillus spp.* in particular *A .flavus (46.5%). A. flavus* was the commonest isolate in AFS(55%), Fungal ball(100%) and CGFRS. (25%).In CIFRS, however, *Rhizopus spp* were most commmonly isolated(50%).

TABLE 9

MINIMUM INHIBITORY CONCENTRATION OF AMPHOTERICIN B TO DIFFERENT MOULDS BY BROTH DILUTION METHOD

SPECIES	NUMBER	SENSITIVE (MIC<2µg/ml)*	RESISTANT (MIC >2µg/ml)	MEAN MIC (µg/ml)
ATCC A.flavus 204304	1	1 (100%)	-	0.5
A.flavus	20	20 (100%)	-	0.5
Rhizopus spp	6	6 (100%)	-	0.5
A.fumigatus	5	5 (100%)	-	0.25
A.niger	3	3 (100%)	-	0.125
A.nidulans	1	1 (100%)	-	1
A.clavatus	1	1 (100%)	-	1
Penicillium spp.	1	1 (100%)	-	0.5
Paecilomyces variotii	1	1 (100%)	-	0.25

*Interpretive criteria currently not standardized .studies show that MICs above 2µg/ml are associated with treatment failure and < 2µg/ml with clinical cure. All the isolates in the study were sensitive to Amphotericin B and were in the MIC range of 0.25 to 1µg/ml.

TABLE 10

MINIMUM INHIBITORY CONCENTRATION OF ITRACONAZOLE TODIFFERENT MOLDS BY BROTH DILUTION METHOD

SPECIES	NUMBER	SENSITIVE (MIC<8µg/ml)*	RESISTANT (MIC >8µg/ml)	MEAN MIC (µg/ml)
ATCC A.flavus 204304	1	1 (100%)	-	0.25
A.flavus	20	20 (100%)	-	0.125
Rhizopus spp	6	6 (100%)	-	0.25
A.fumigatus	5	5 (100%)	-	0.0313
A.niger	3	3 (100%)	-	0.125
A.nidulans	1	1 (100%)	-	0.5
A.clavatus	1	1 (100%)	-	0.25
Penicillium spp.	1	1 (100%)	-	0.125
Paecilomyces variotii.	1	1 (100%)	-	0.25

*Interpretive criteria currently not standardized .Studies show that MICs above 8 µg/ml are associated with treatment failure and < 8 µg/ml with clinical cure.

All isolates were universally sensitive to Itraconazole by broth dilution method and were in the MIC range of 0.0313 to 0.5.

TABLE 11

DISK DIFFUSION FOR FILAMENTOUS FUNGI FOR AMPHOTERICIN B

SPECIES	NUMBER	S(ZONE>15 mm)	SDD/I(Zone 13-14 mm)	R(Zone<12 mm)
ATCC A.flavus204304	1	1 (100%)	-	-
A.flavus	20	15 (75%)	4 (20%)	1(5%)
Rhizopus spp	6	6 (100%)	-	-
A.fumigatus	5	5 (100%)	-	-
A.niger	3	3 (100%)	-	-
A.nidulans	1	1 (100%)	-	-
A.clavatus	1	1 (100%)	-	-
Penicillium spp.	1	1 (100%)	-	-
Paecilomyces variotii	1	1 (100%)	-	-

S=SUSCEPTIBLE;SDD=SUSCEPTIBLE DOSE DEPENDENT;R=RESISTANT

75 % of the *A.flavus* were sensitive to Amphotericin B, 5 % were resistant and 20% were susceptible dose dependent. All other species were universally sensitive to Amphotericin B.

TABLE 12

DISK DIFFUSION FOR FILAMENTOUS FUNGI FOR ITRACONAZOLE

SPECIES	NUMBER	S (ZONE> 17 mm)	SDD/I (Zone 14-16 mm)	RESISTANT (Zone<13mm)
ATCC A.flavus 204304	1	1 (100%)	-	-
A.flavus	20	18 (90%)	2(10%)	-
Rhizopus spp	6	6 (100%)	-	-
A.fumigatus	5	4 (80%)	1(20%)	-
A.niger	3	3 (100%)	-	-
A.nidulans	1	1 (100%)	-	-
A.clavatus	1	1 (100%)	-	-
Penicillium spp.	1	1 (100%)	-	-
Paecilomyces variotii	1	1 (100%)	-	-

Of the20 *Aspergillus spp.*90% were sensitive to Itraconazole and 10% were susceptible dose dependent. 80 % of *A.fumigatus* was sensitive to Itraconazole and 20% was susceptible dose dependent. All other species were sensitive to Itraconazole.

TABLE 13

E TEST FOR AMPHOTERICIN B FOR FILAMENTOUS FUNGI

SPECIES	NUMBER	AMPHOTERICIN		
		S	R	MEAN MIC (µg/ml)
ATCC A.flavus 204304	1	1 (100%)	-	0.5
A.flavus	20	20 (90%)	-	0.25
Rhizopus spp	6	6 (100%)	-	0.5
A.fumigatus	5	5 (100%)	-	0.0313
A.niger	3	3 (100%)	-	0.0313
A.nidulans	1	1 (100%)	-	1
A.clavatus	1	1 (100%)	-	1
Penicillium spp.	1	1 (100%)	-	0.5
Paecilomyces variotii	1	1 (100%)	-	0.0625

All the isolates were sensitive to Amphotericin B by Epsilometer test and MIC range was from 0.0313 to 1.

TABLE 14

AGAR DILUTION MIC FOR ITRACONAZOLE AND AMPHOTERICIN B

SPECIES	NO.	AMPHOTERICIN B			ITRACONAZOLE		
		S	R	MEAN MIC (µg/ml)	S	R	MEAN MIC (µg/ml)
ATCC A.flavus 204304	1	1 (100%)	-	0.5	1 (100%)	-	0.25
A.flavus	20	20 (100%)	-	0.5	20 (100%)	-	0.125
Rhizopus spp	6	6 (100%)		0.5	6 (100%)	-	0.25
A.fumigatus	5	5 (100%)	-	0.25	5 (100%)	-	0.0313
A.niger	3	3 (100%)	-	0.125	3 (100%)	-	0.125
A.nidulans	1	1 (100%)	-	1	1 (100%)	-	0.5
A.clavatus	1	1 (100%)	-	1	1 (100%)	-	0.25
Penicillium spp.	1	1 (100%)	-	0.5	1 (100%)	-	0.125
Paecilomyces variotii	1	1 (100%)	-	0.25	1 (100%)	-	0.25

All isolates were sensitive to Itraconazole and Amphotericin B by agar dilution method.

TABLE 15

COMPARISON OF DISK DIFFUSION, E TEST AND AGAR DILUTION FOR AMPHOTERICIN B WITH MICROBROTH REFERENCE METHOD

SPECIES (N)	Broth dilution (S)	Disk diffusion			E test		Agar dilution	
		M	M	VM	M	VM	M	VM
ATCC A.flavus 204304(1)	1	-	-	-	-	-	-	-
A.flavus(20)	20	4 **(20 %)**	1 (**5%**)	-	-	-	-	-
Rhizopus spp(6)	6	-	-	-	-	-	-	-
A.fumigatus(5)	5	-	-	-	-	-	-	-
A.niger(3)	3	-	-	-	-	-	-	-
A.nidulans(1)	1	-	-	-	-	-	-	-
A.clavatus(1)	1	-	-	-	-	-	-	-
Penicillium spp.(1)	1	-	-	-	-	-	-	-
Paecilomyces variotii(1)	1	-	-	-	-	-	-	-

m=minor error; M=Major error ; VM=Very major error

Minor error: Shifts between susceptible and susceptible dose dependent or between resistant and susceptible dose dependent.

Major error: Isolate resistant by other methods but susceptible by broth Dilution.

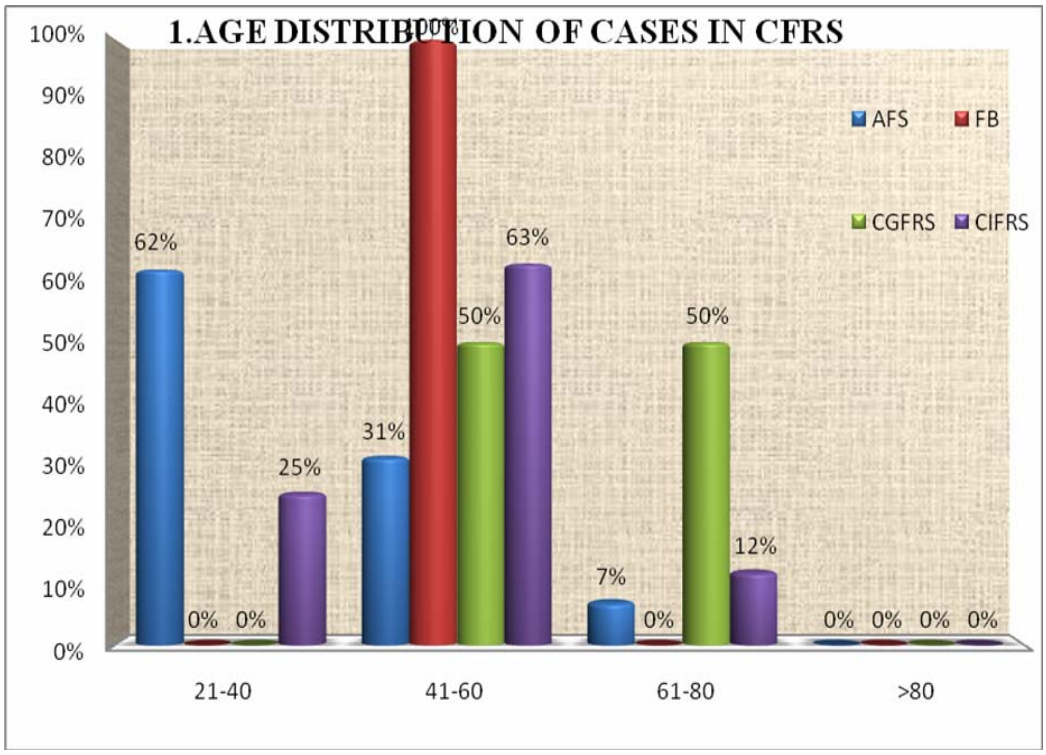
Very major error: Broth dilution shows resistance and others show as sensitive

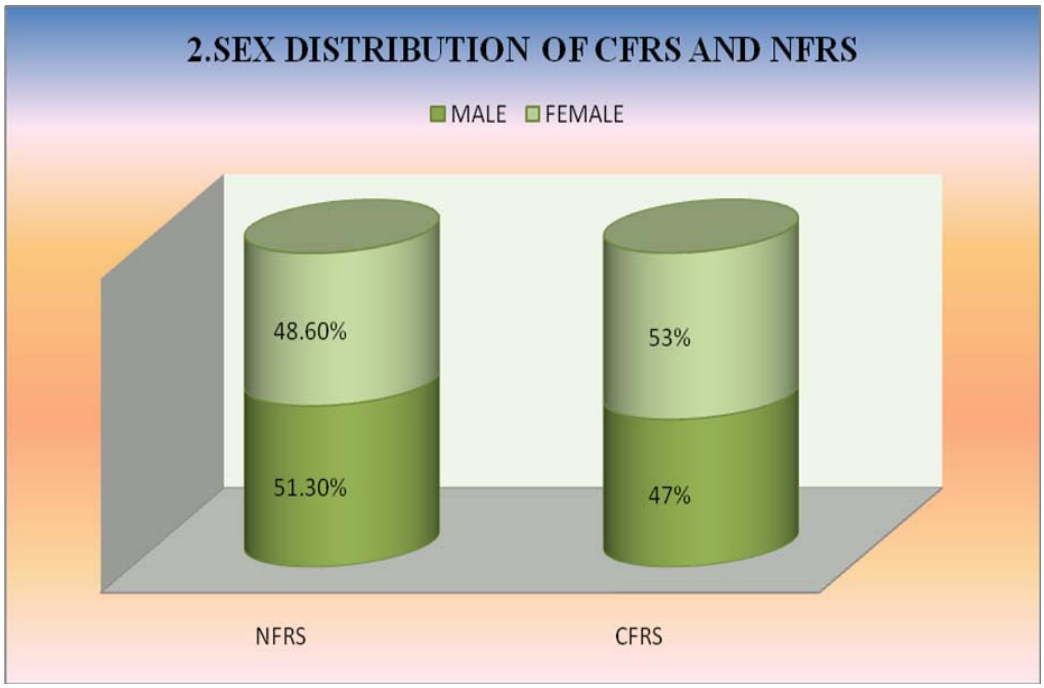
TABLE 16

COMPARISON OF DISK DIFFUSION, AND AGAR DILUTION FOR ITRACONAZOLE WITH MICROBROTH REFERENCE METHOD

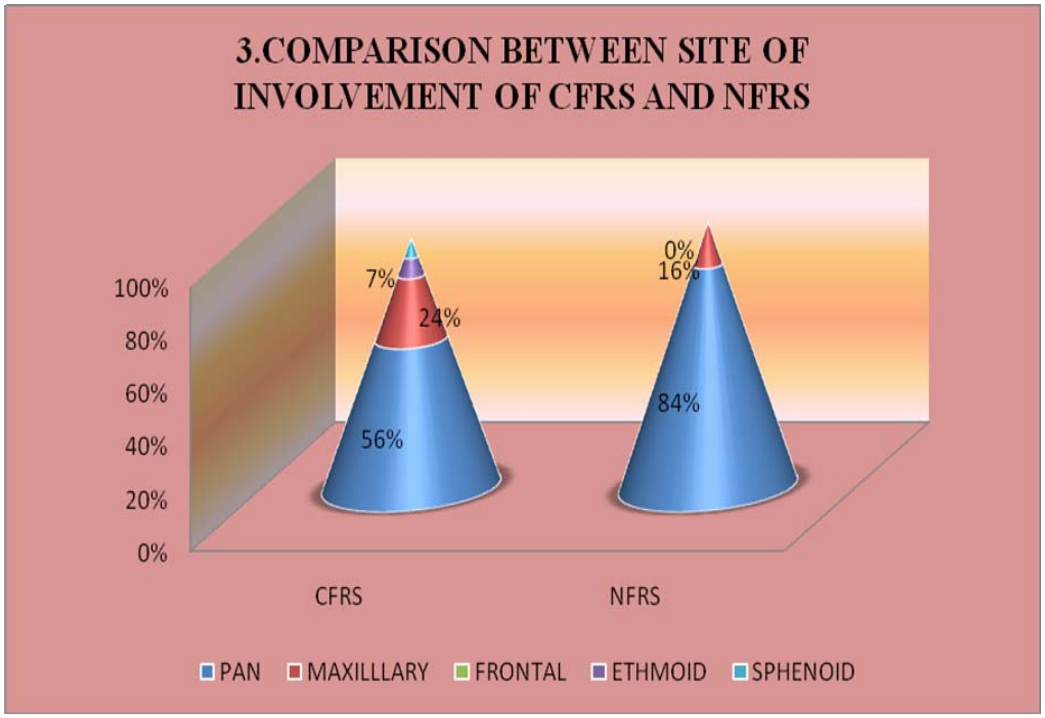
SPECIES	Broth dilution (S)	Disk diffusion			Agar dilution	
		M	M	VM	M	VM
ATCC A.flavus 204304(1)	1	-	-	-	-	-
A.flavus (20)	20	2 (10%)	-	-	-	-
Rhizopus spp(6)	6	-	-	-	-	-
A.fumigatus (5)	5	1 (20%)	-	-	-	-
A.niger (3)	3	-	-	-	-	-
A.nidulans (1)	1	-	-	-	-	-
A.clavatus (1)	1	-	-	-	-	-
Penicillium spp.(1)	1	-	-	-	-	-
Paecilomyces variotii (1)	1	-	-	-	-	-

m=minor error; M=Major error; VM=Very major error.

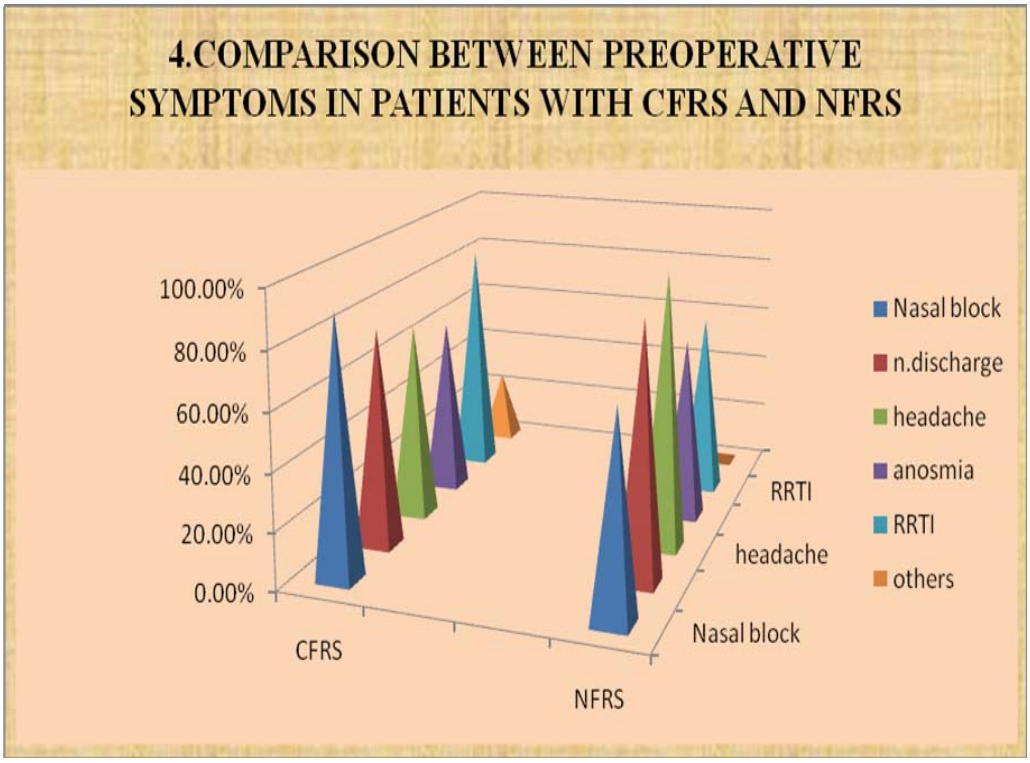

1.AGE DISTRIBUTION OF CASES IN CFRS
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
AFS
FB
CGFRS
CIFRS
62%
0%
0%
25%
31%
100%
50%
63%
7%
0%
50%
12%
0%
0%
0%
0%
21-40
41-60
61-80
>80


2.SEX DISTRIBUTION OF CFRS AND NFRS
MALE
FEMALE
48.60%
51.30%
53%
47%
NFRS
CFRS

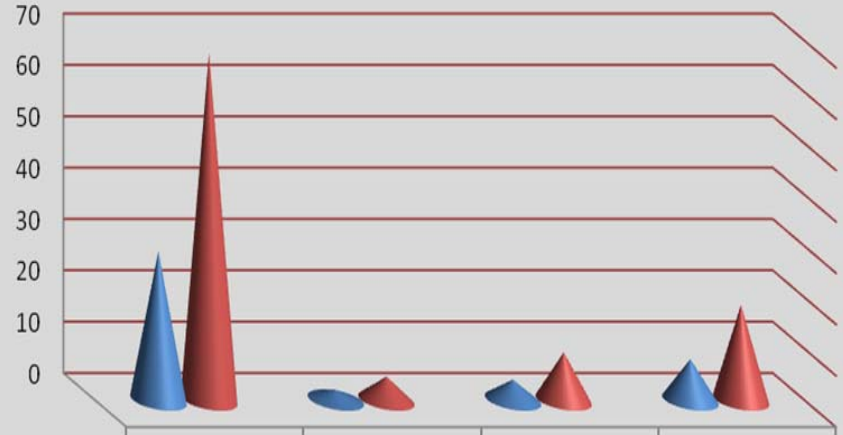
P=0.522(not significant)



P=0.013

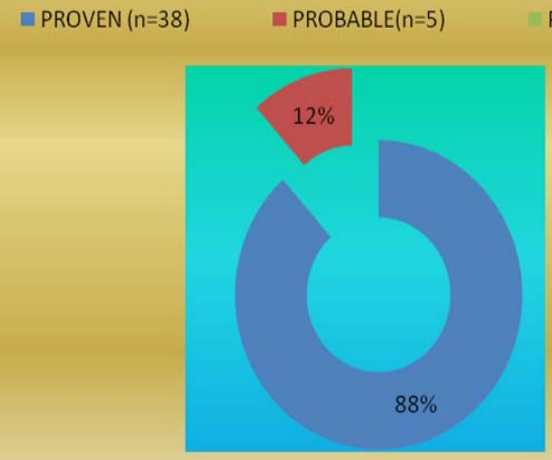


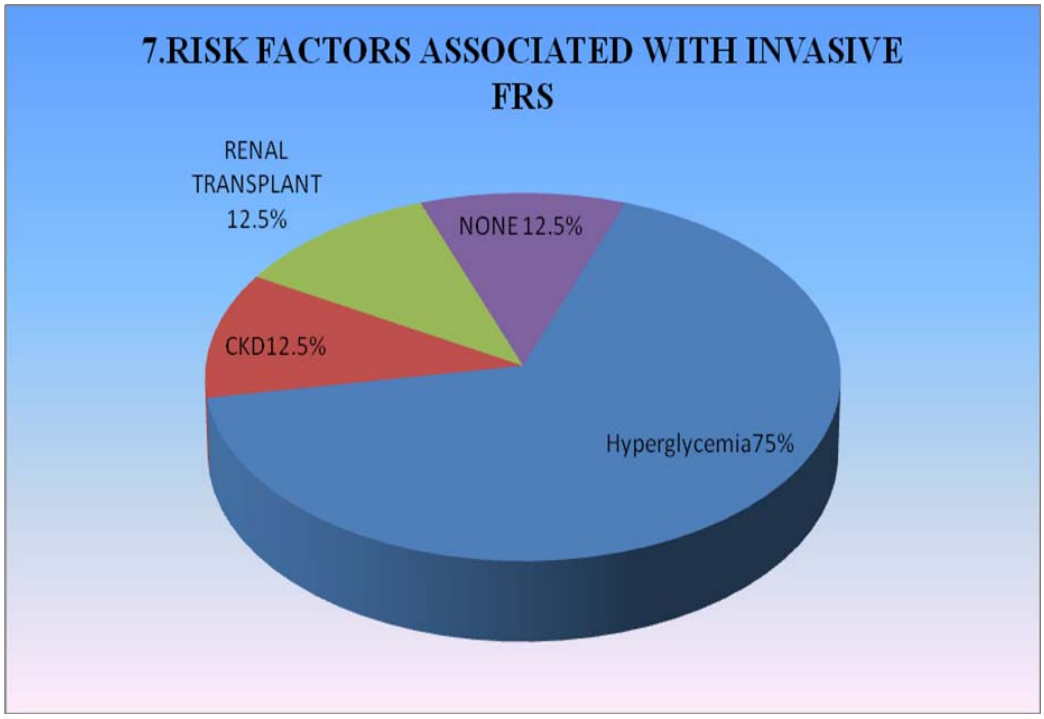
5.CATEGORISATION OF THE CASES OF FUNGAL SINUSITIS

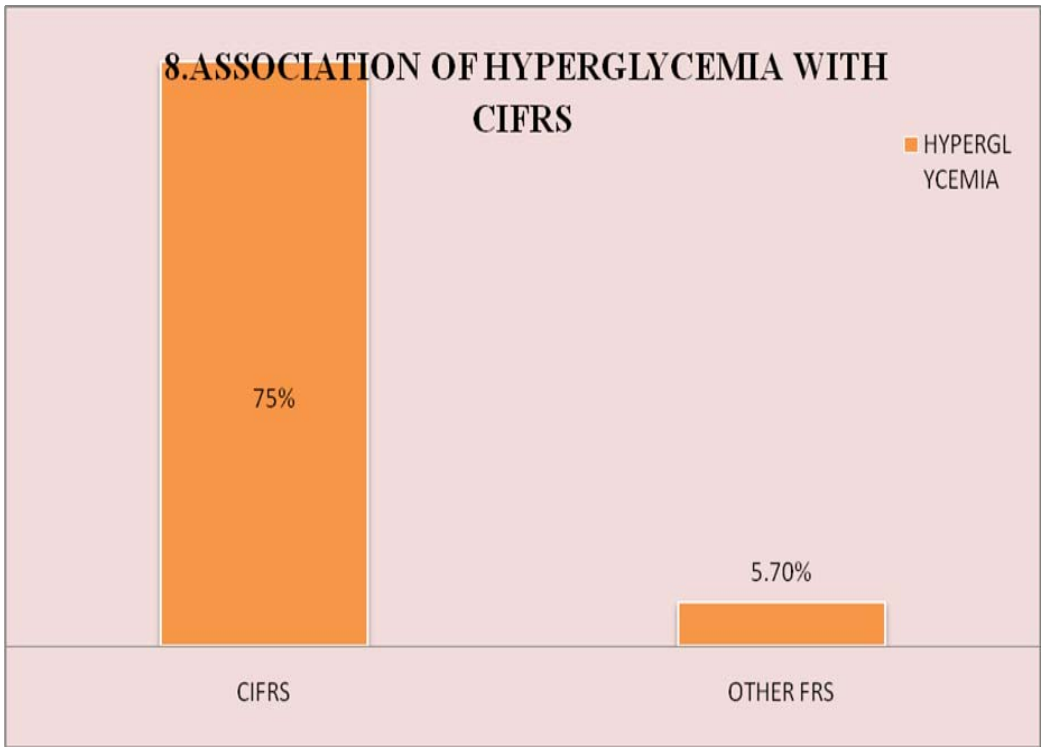


	AFRS	FUNGAL BALL	CGFRS	CIFRS
NUMBER	29	2	4	8
%	67.77	4.6	9.3	18.6

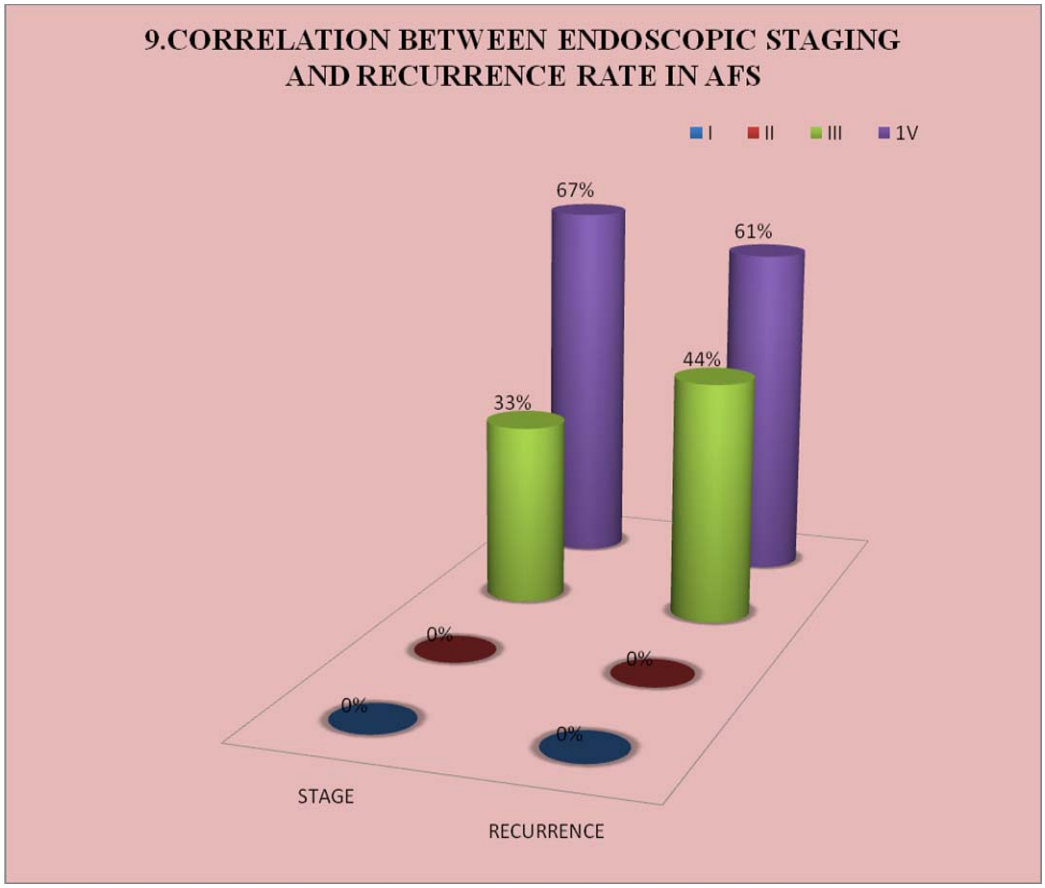
6.CLASSIFICATION OF INVASIVE FUNGAL INFECTIONS

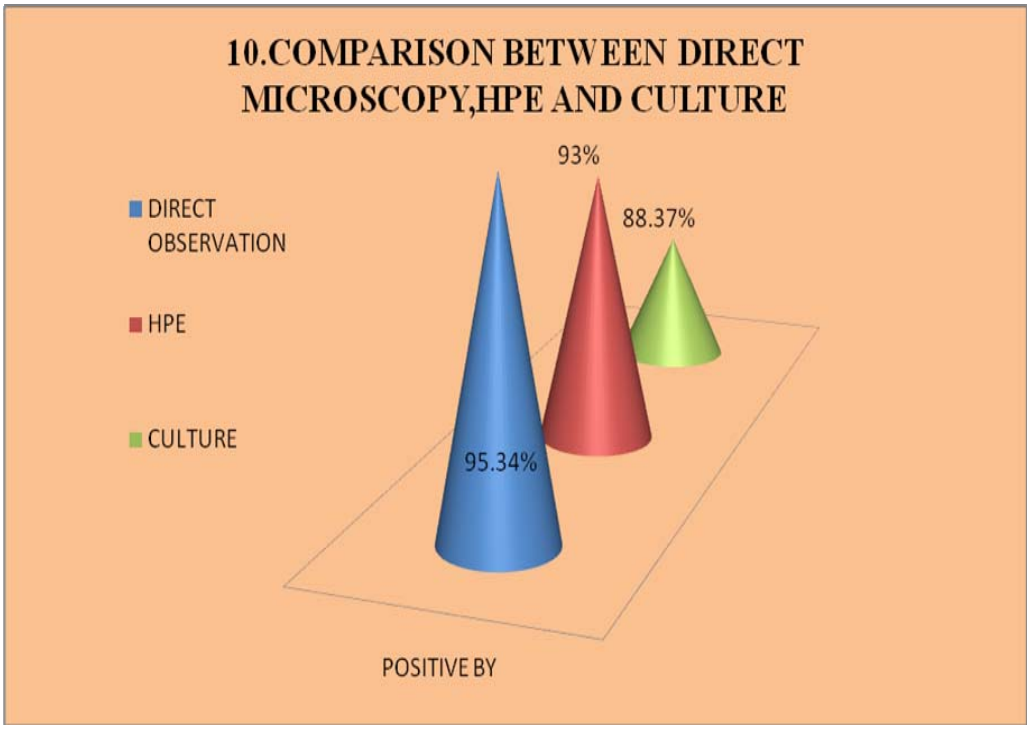


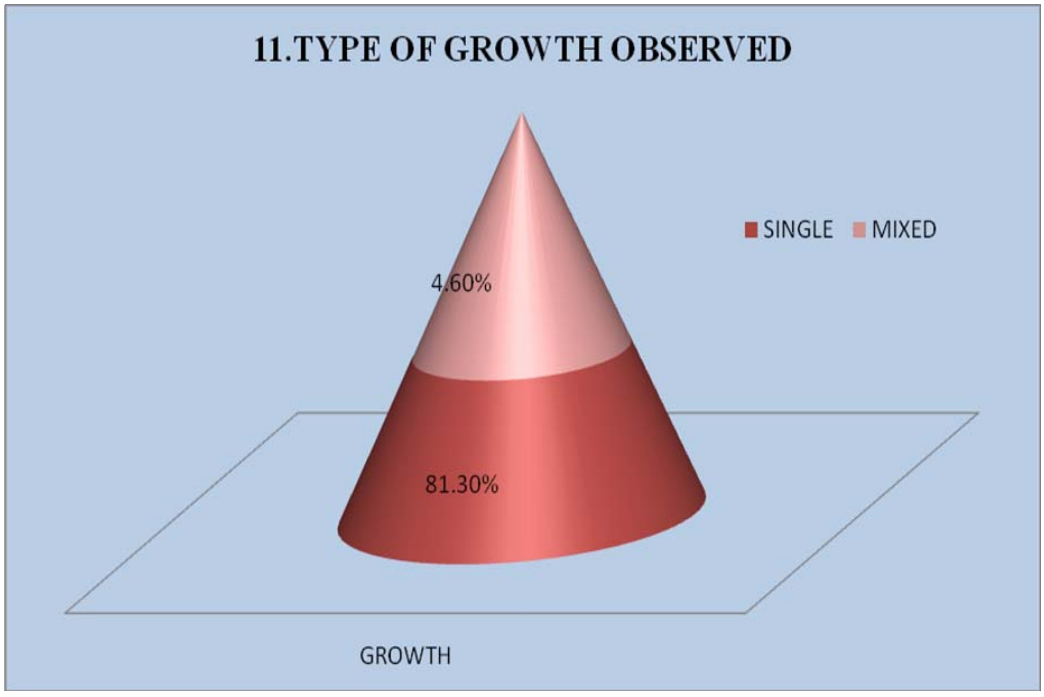

7.RISK FACTORS ASSOCIATED WITH INVASIVE FRS
RENAL TRANSPLANT 12.5%
NONE 12.5%
CKD12.5%
Hyperglycemia75%

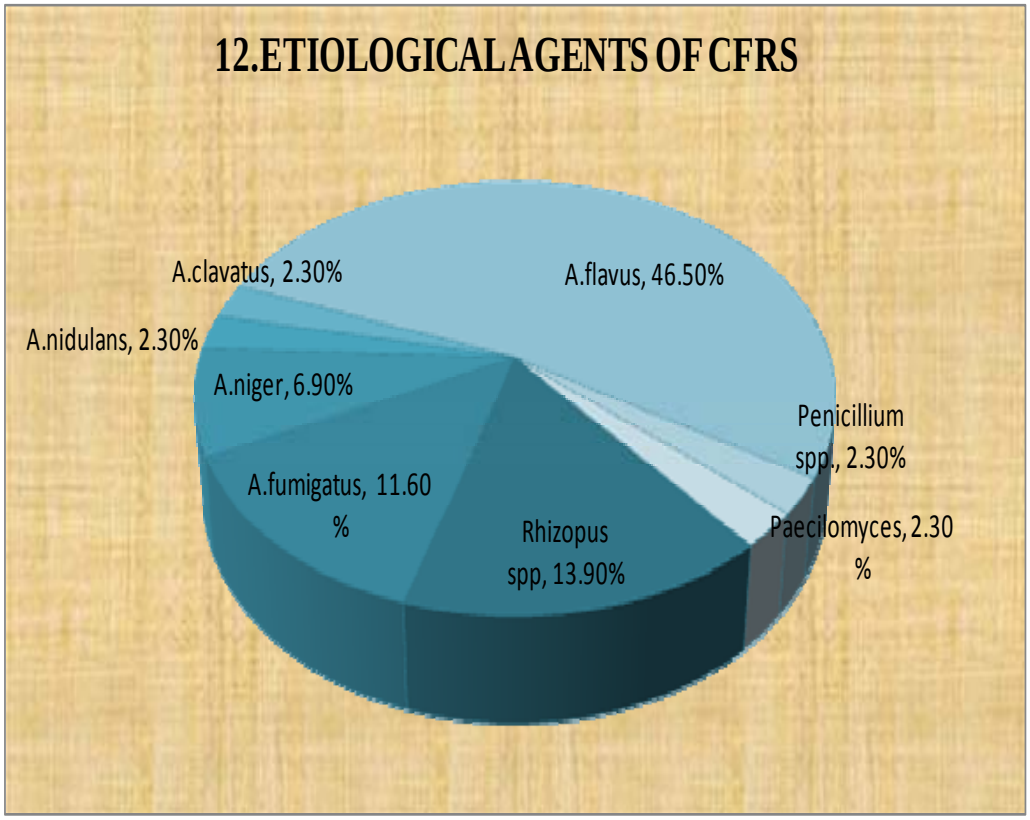

8.ASSOCIATION OF HYPERGLYCEMIA WITH CIFRS
HYPERGLYCEMIA
75%
5.70%
CIFRS
OTHER FRS

P=0.002


9.CORRELATION BETWEEN ENDOSCOPIC STAGING AND RECURRENCE RATE IN AFS
I
II
III
1V
67%
61%
44%
33%
0%
0%
0%
0%
STAGE
RECURRENCE

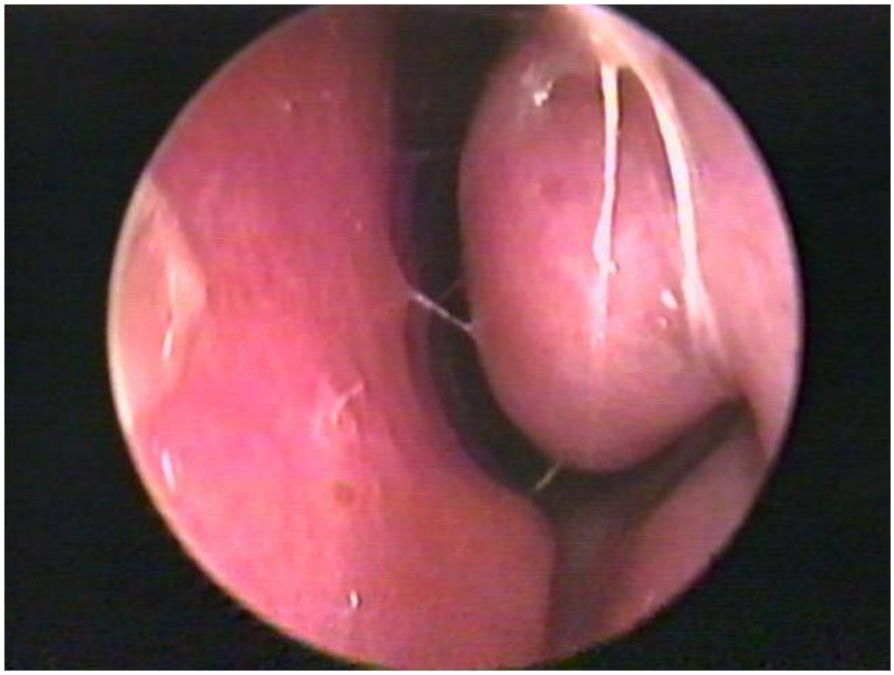

10.COMPARISON BETWEEN DIRECT MICROSCOPY,HPE AND CULTURE
93%
88.37%
DIRECT OBSERVATION
HPE
CULTURE
95.34%
POSITIVE BY


11.TYPE OF GROWTH OBSERVED
SINGLE
MIXED
4.60%
81.30%
GROWTH

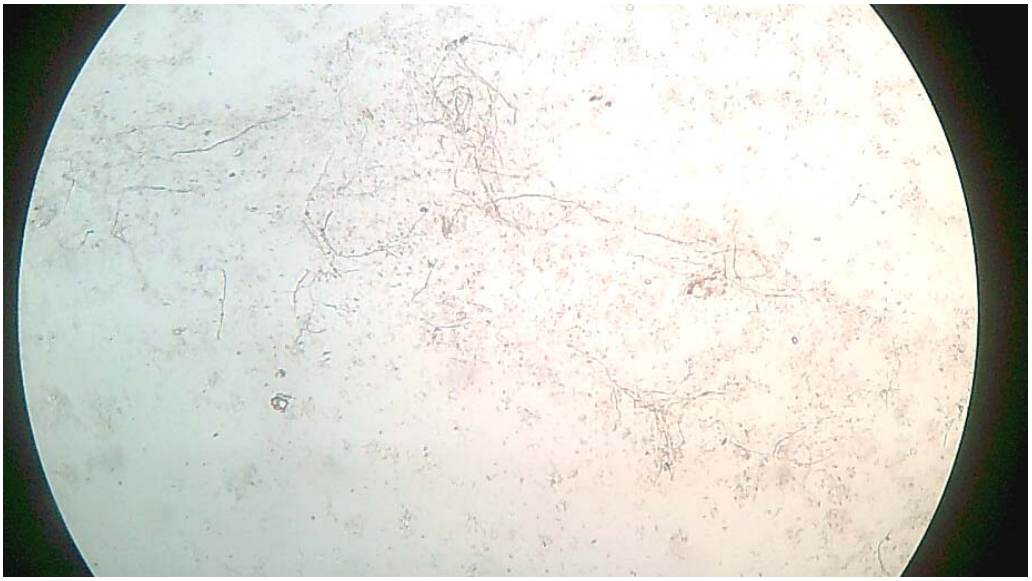

12.ETIOLOGICAL AGENTS OF CFRS
A.clavatus, 2.30%
A.flavus, 46.50%
A.nidulans, 2.30%
A.niger, 6.90%
Penicillium spp., 2.30%
A.fumigatus, 11.60 %
Rhizopus spp, 13.90%
Paecilomyces, 2.30 %

PATIENT WITH CGFRS : NOTE THE GRANULOMATOUS SWELLING IN LEFT MAXILLA

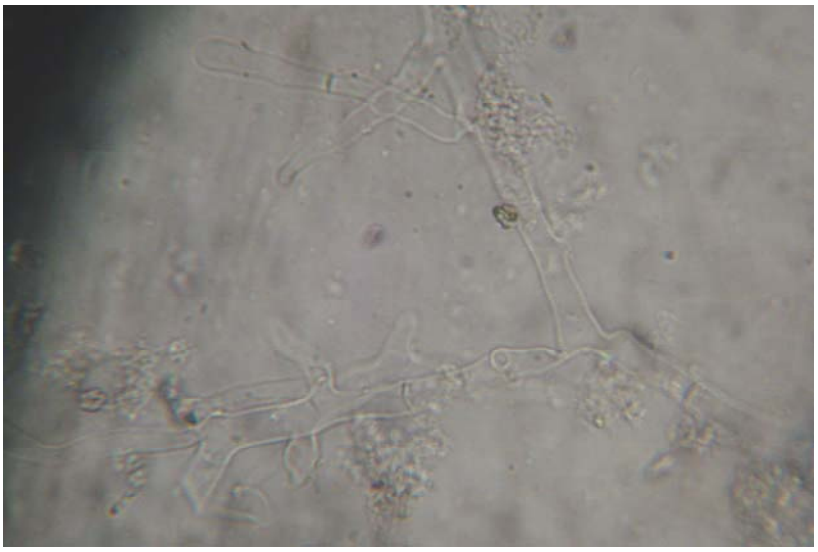
CT SCAN SHOWING LEFT MAXILLARY SIMUSITIS NOTE SINUS WALL EROSION



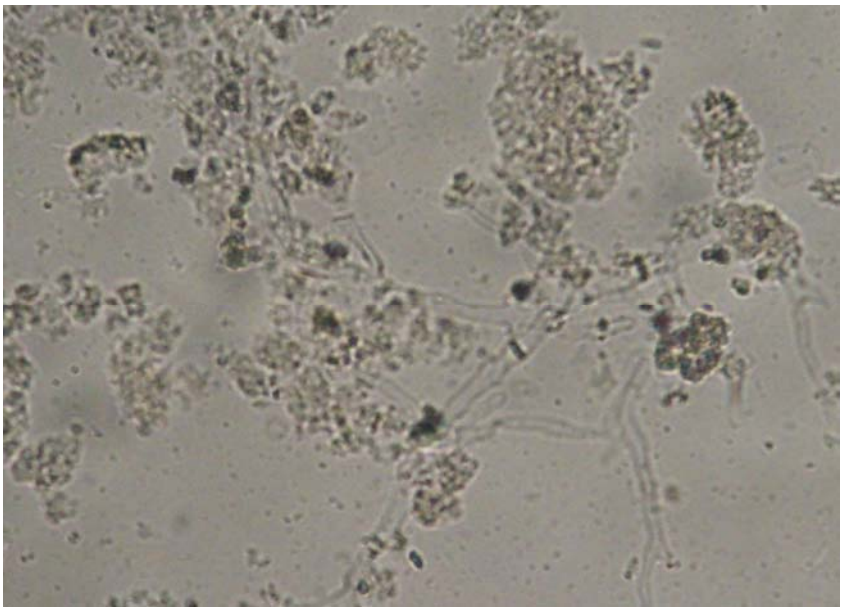
FESS SHOWING ALLERGIC MUCIN WITH POLYP.



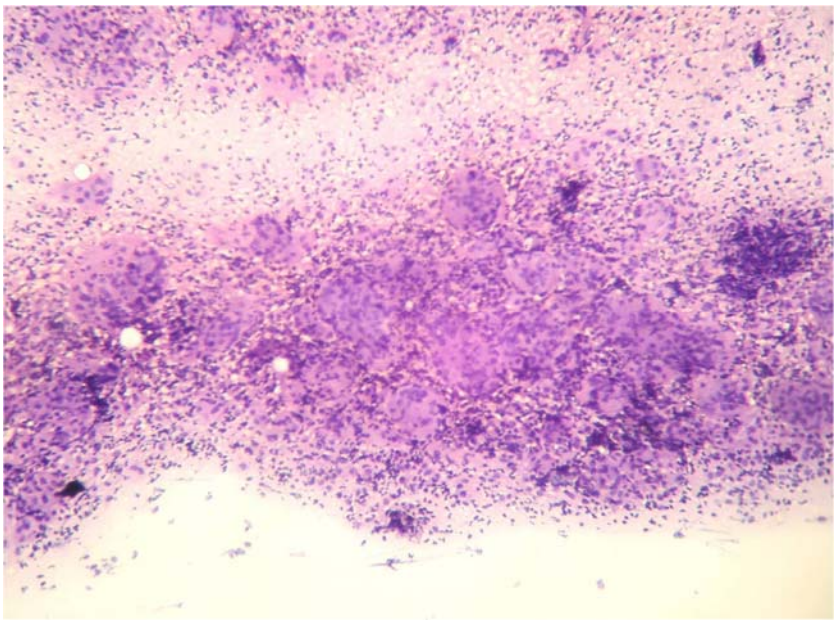
10X MAGNIFICATION OF 10% KOH SHOWING HYPHAL ELEMENTS



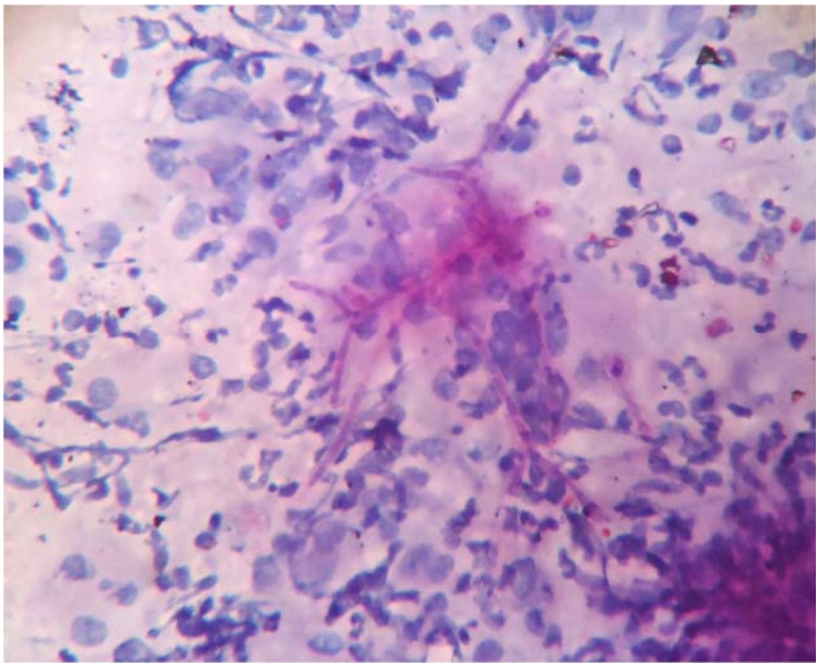
(40X MAGNIFICATION) 10% KOH SHOWING BROAD PAUCISEPTATE HYPHAE WITH OBTUSE ANGLE BRANCHING



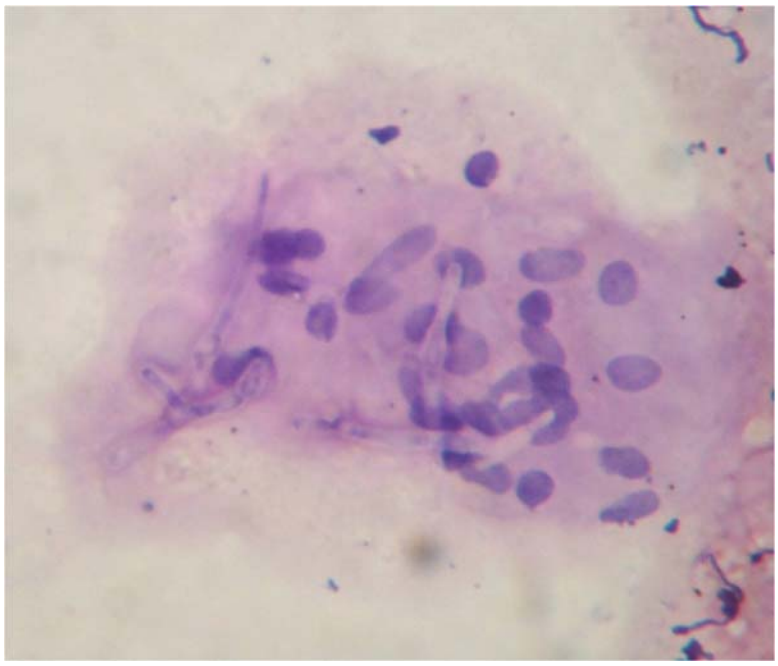
10% KOH SHOWING SLENDER SEPTATE HYPHAE WITH ACUTE ANGLE BRANCHING



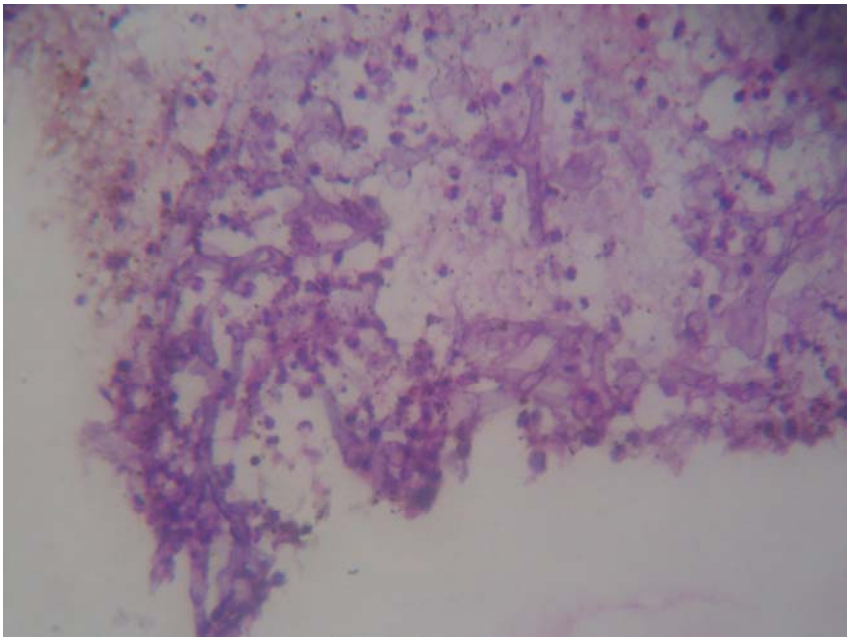
H&E SECTION SHOWING GRANULOMATOUS REACTION



PAS SECTION SHOWING SEPTATE SLENDER HYPHAE WITH ACUTE ANGLE BRANCHING



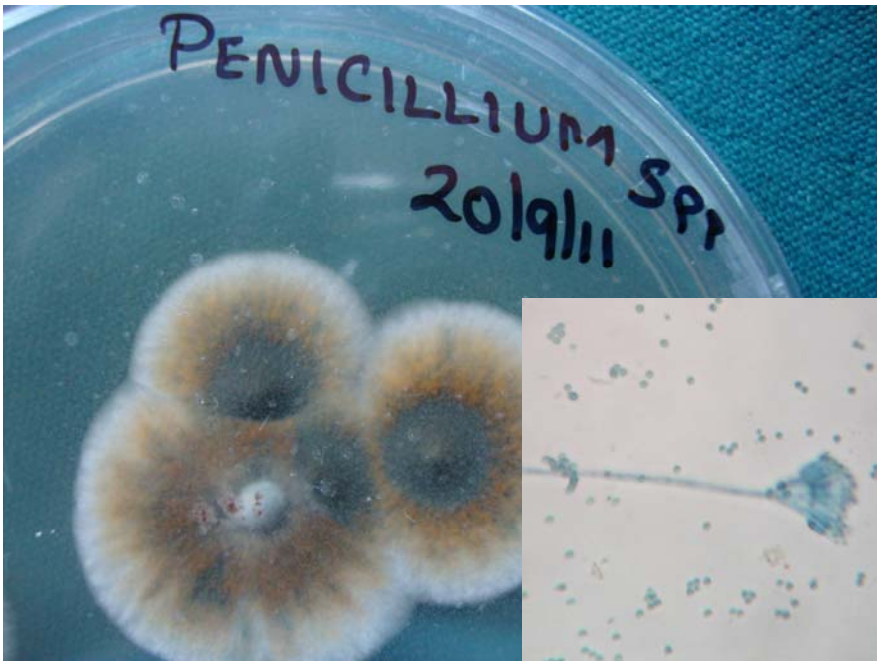
H&E SHOWING HYPHAL FORMS



GIEMSA STAIN OF A FUNGAL BALL

Aspergillus niger **(INSERTMICROSCOPIC PICTURE)**

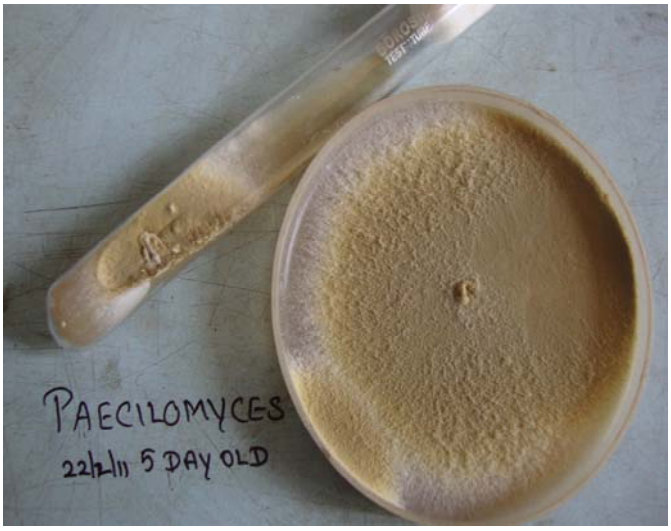
Aspergillus flavus **(INSERTMICROSCOPIC PICTURE)**



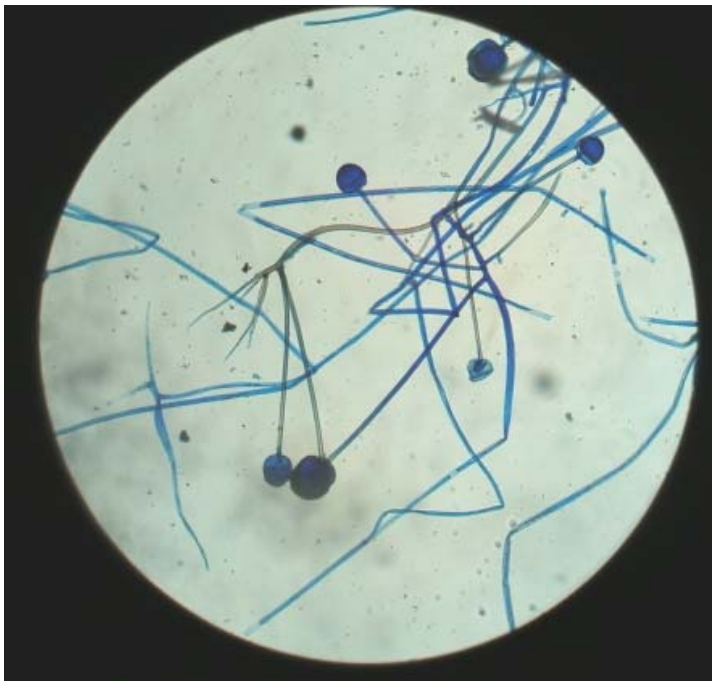
Penicillium spp **(INSERTMICROSCOPIC PICTURE)**

Aspergillus clavatus **(INSERTMICROSCOPIC PICTURE)**

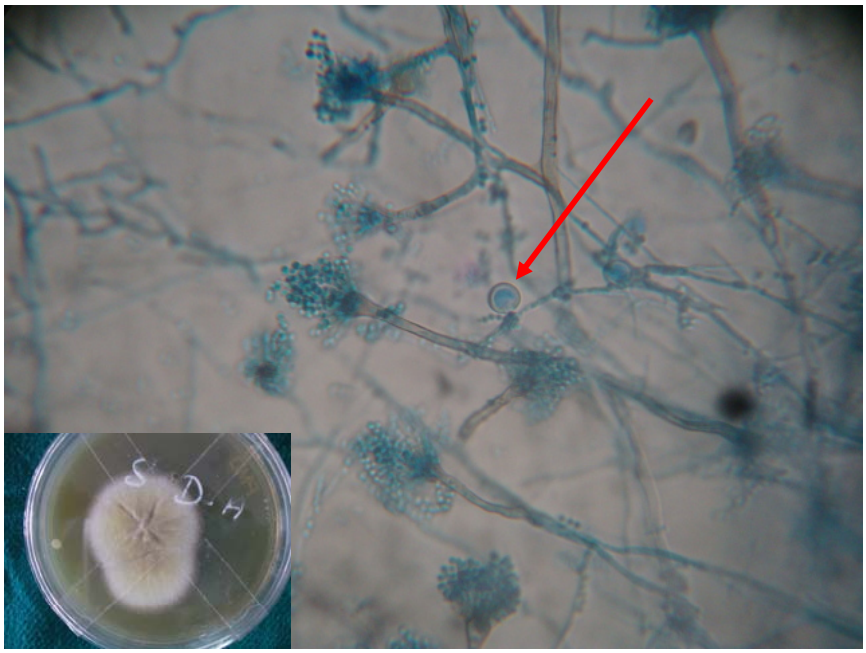
Aspergillus versicolor



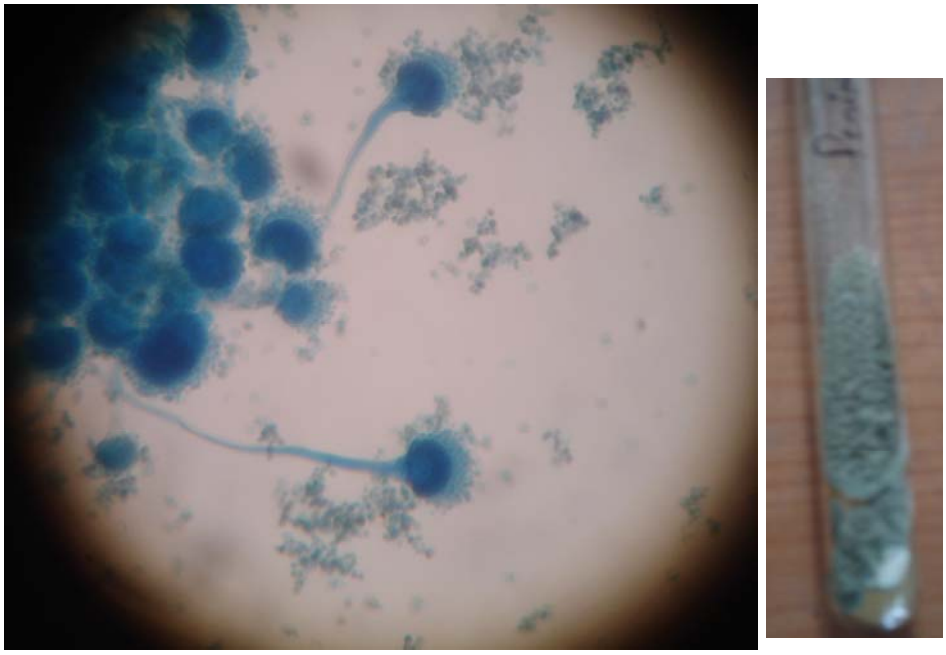
Paecilomyces variotii



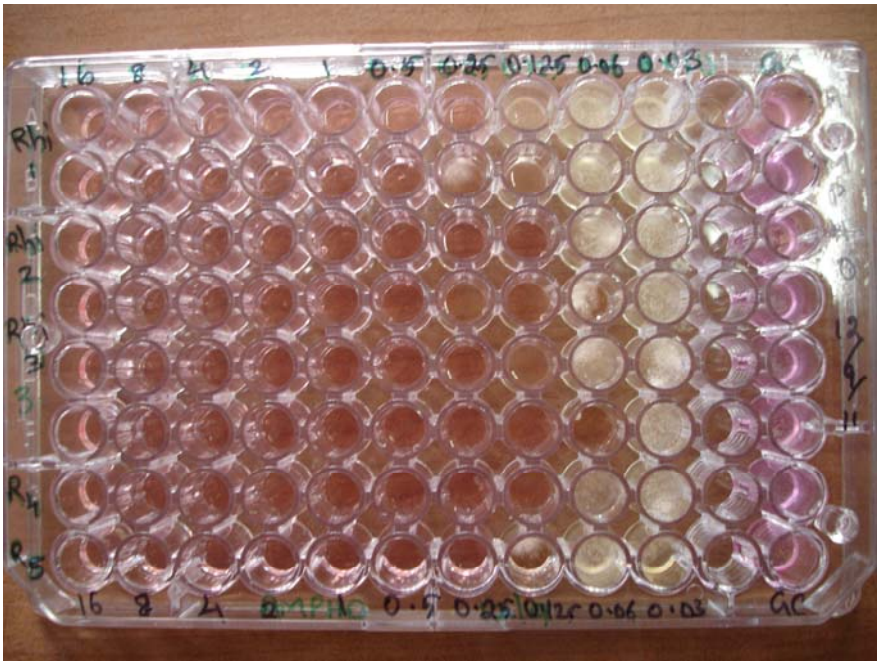
Rhizopus spp



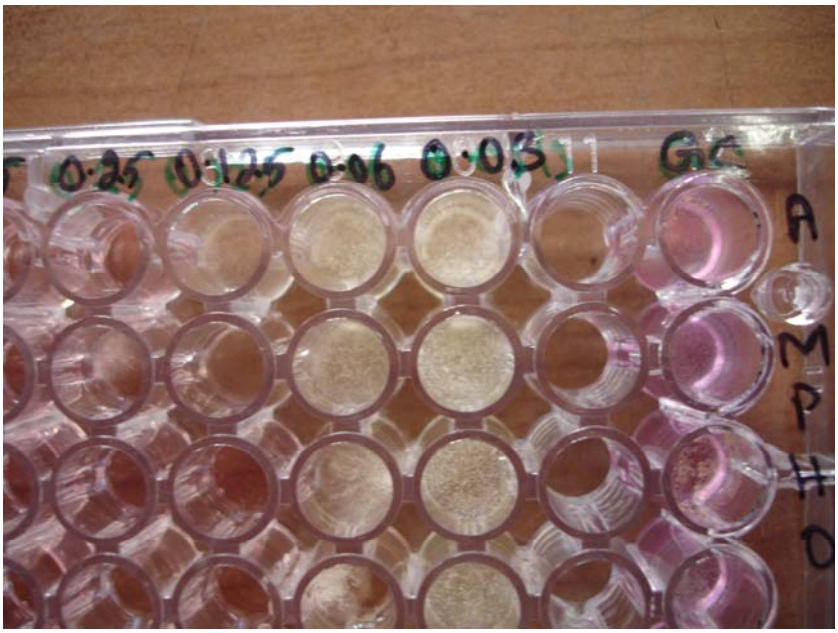
Aspergillus nidulans **(NOTE : HULLE CELLS)**



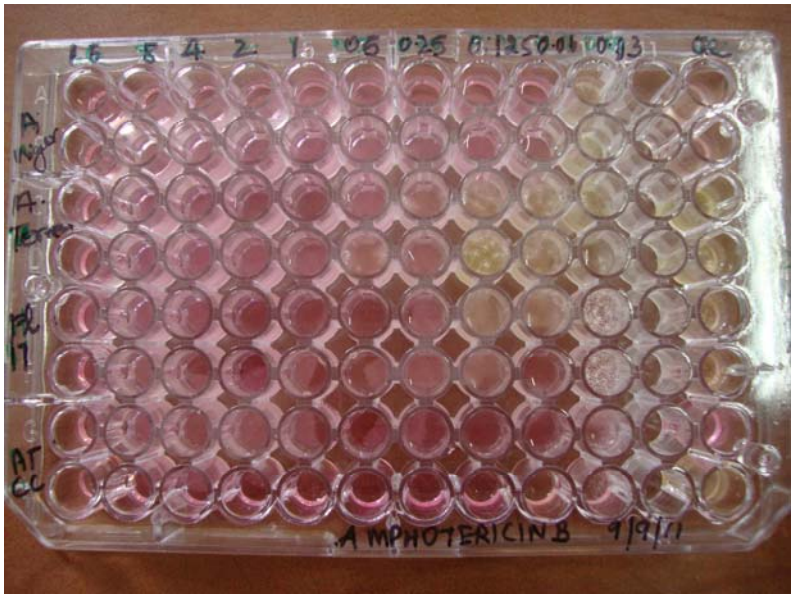
Aspergillus fumigatus



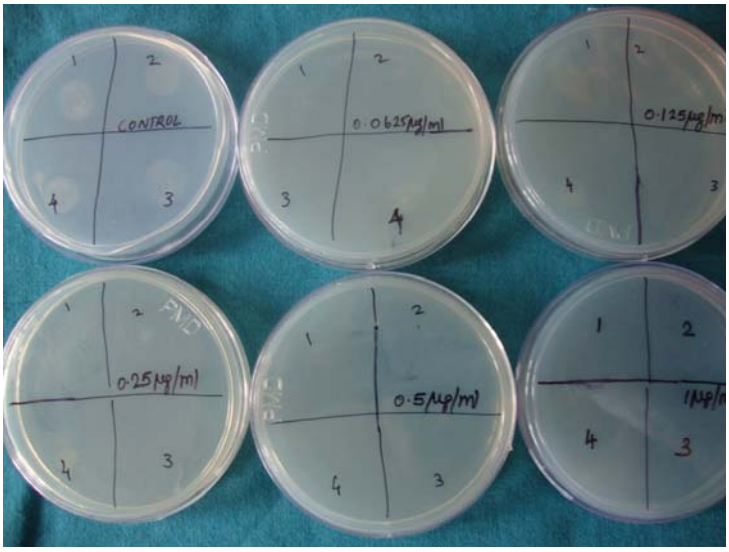
MICROBROTH DILUTION FOR ITRACONAZOLE



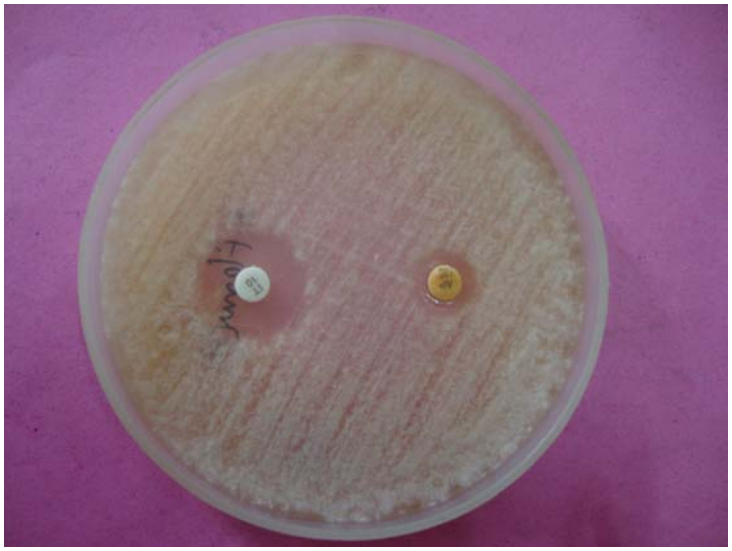
MIC BREAK POINT DETERMINATION



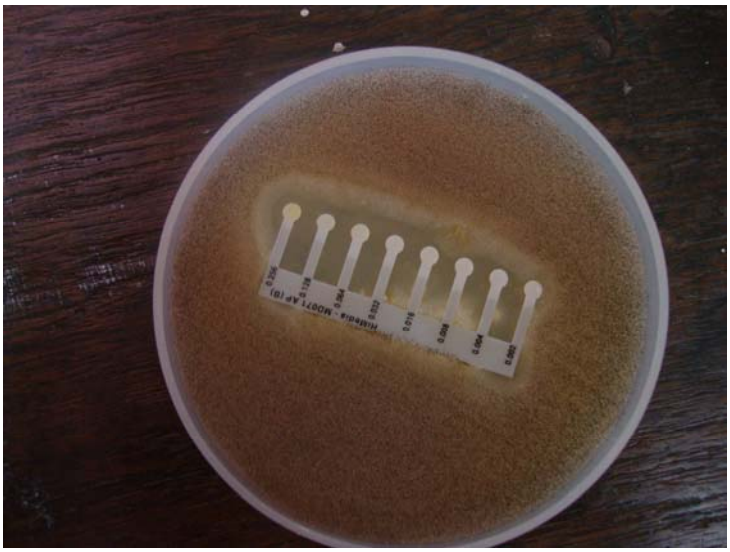
MIC DETERMINATION FOR AMPHOTERICIN B



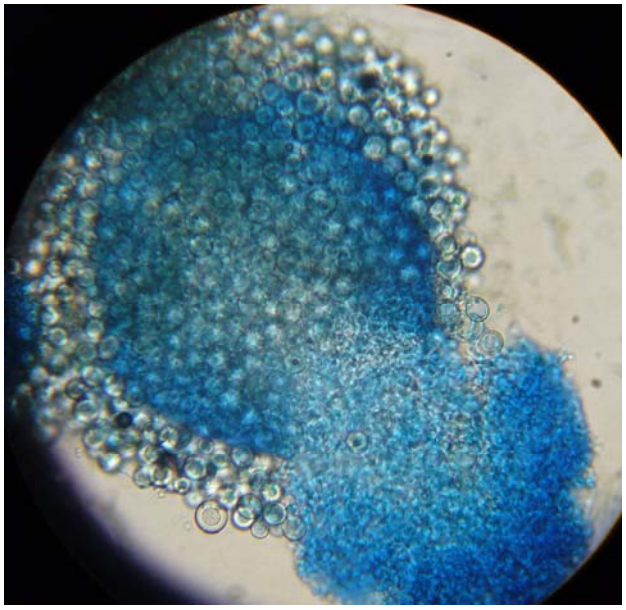
AGAR DILUTION SUSCEPTIBILITY TESTING



DISK DIFFUSION FOR ITRACONAZOLE AND AMPHOTERICIN B



E TEST FOR *Aspergillus niger*



CLEISTOTHECIA OF *A.nidulans*

DISCUSSION

Fungal Rhinosinusitis is a increasingly recognized entity among cases of chronic rhinosinusitis.The importance is increasing due to the morbidity and mortality caused by FRS.This study was conducted among 380 cases of Chronic Rhinosinusitis who underwent Functional endoscopic sinus surgery and Diagnostic nasal endoscopy at the Upgraded Institute of Otorhinolaryngology during the study period from January 2010 to June 2011. 80 cases which fulfilled the inclusion criteria were included in the study. Of the 80 cases, 43 cases were recognized as chronic Fungal Rhinosinusitis. Overall incidence of FRS was 11.3%. Shiv sekar chatterjee et al, 2009 have recorded an incidence of FRS to be 5-15 %[6]

Of the total 43 cases of fungal sinusitis(table 1),22(51%)were in the age group of 21-40(young adults).An almost equal number 18 patients (41.8%) were in the age group of 41-60(middle age) and a minor number of patients(3) in the age group>60(6.9%).AFS predominated in the 21-40 age group(62%) .This is similar to the observations made by Carrie A Roller et al and Chakrabarti et al who have observed that the disease predominated in young adults[53,54]. This can be attributed to the fact that young adults who commonly go to the field frequently get mucosal injuries of paranasal sinuses and acquire the agent from the area of work, travel and ecology.In contrast to the popular belief that AFS is commonly observed in hot and dry climate,it is now reported more in hot and humid climate of south India[105,106].CIFRS predominated in the 41-60 age group (63%).All cases of Fungal ball and Chronic granulomatous fungal sinusitis were clustered in the age group of 41-60(middle age) .

Shivsekar chatterjee et al, 2009 has also made similar observations that invasive sinusitis is more common in middle aged and elderly due to high prevalence of risk factors like diabetes.[6] In contrast, maximum number of cases of non fungal sinusitis were in the age group of 41-60(54%).

A Slight female predominance was noted in sinusitis of fungal etiology (55.8%) whereas males predominated in non FRS group (51.4%) (Table 1).However this gender difference was not statistically significant. **(p=0.522**). This is in contrast to the observations made by Shiv sekar et al [6]2009who observed a male predominance in developing CFRS.

Majority of patients of CFRS presented with nasal block (90.6%) as the predominant complaint(Table 2) ,though Headache predominated as the presenting complaint(97%) in NFRS. Headache was the presenting complaint in only 69.7 % of the people with fungal sinusitis. Symptoms like proptosis, facial puffiness, tinnitus, diminished vision, altered sensorium, speech disturbances and nerve palsies were more common in CFRS than in nonfungal CRS probably due to the erosive and invasive nature of the causative fungi. Recurrent respiratory tract infection was seen in 83.7% and 65% of CFRS and FRS of non fungal etiology. This differs slightly from the study done by Tekin Karsligil et al[25],2008 where headache and nasal block both predominated in the CFRS group (93.9%) . Other throat and laryngeal complications were present more in FRS than non FRS(63.6%)[25].This data is similar to the observations made in our study.

In Non FRS group, 31 cases, (84%) involved all sinuses,i.e,pansinusitis. (Table 3)In contrast, only 58% was pansinusitis in CFRS group. The next common was bilateral Maxillary sinusitis (14%).Bilateral ethmoid and bilateral sphenoid sinus alone was involved in 7% of cases each. Unilateral distribution was seen only in case of maxillary sinus which constituted 14.3 % of cases. In statistical analysis**, $p<0.05$** was obtained. So, it is a statistically significant fact that NFRS commonly presented as pansinusitis whereas FRS can also affect only a single sinus often .Similar findings have been recorded by Tekin Karsligil et al[25]2008 and Shiv sekar chatterjee et al[6],2009 who have recorded pansinusitis as the commonest occurrence (79%) in cases of FRS. Tajen et al[107] have also observed that fungi may cause inflammation in even only one sinus also.

Among the cases of proven chronic fungal rhinosinusitis(n=43), 29 cases ,i.e, 67% were allergic fungal sinusitis,2 cases,i.e 5 % were fungal ball ,4 cases,i.e,9% were chronic granulomatous sinusitis and 8 cases ,i.e,19 % were chronic invasive sinusitis .(Table 4) Occurrence of Allergic fungal sinusitis predominated (67%) when compared to other categories. This is similar to the study done by Rajiv .C. Michael et al ,2008 who has reported 63% due to allergic fungal disease [22] and Ponikau et al [5] ,1999 who diagnosed allergic FRS in 93% of the patients by advanced methods of sample collection and processing. This is in contrast to the results by Chakrabarthy et al[60] 1992 and Panda et al [61] ,1998 who have reported lesser numbers as affected by allergic fungal sinusitis. Alphin et al,Houser et al and Schubert et al have observed that the original incidence is not known and that further studies are required for

confirmation .[64,62,63] In the 8 chronic invasive fungal sinusitis, 6,i.e. 75% were proven sinusitis and 2 cases, i.e,25% was Probable fungal sinusitis.(10% KOH positive, culture negative).

In the 43 chronic fungal rhinosinusitis cases, Hyperglycemia was noted as a risk factor in 8 cases(18.6%)(Table 5).When comparing chronic invasive rhinosinusitis and other subtypes, Hyperglycemia was observed as a risk factor in 75% (6 of 8 cases)of Chronic Invasive Rhinosinusitis and the same risk factor was observed in only 6 % of other subtypes**.(p=0.002)** This shows the increased tendency of fungal infections to occur in a more invasive form if there is underlying hyperglycemia due to uncontrolled diabetes. Varying data are available regarding the existence of hyperglycemia as a risk factor for development of the disease. Michael et al,2008 reported uncontrolled diabetes in 38.8% of cases of invasive fungal sinusitis[22] and they have suggested that the study population may have undiagnosed diabetes mellitus since diabetes is known to be extremely common in India. Mohapatra et al [65], 2010, has observed that hyperglycemia was noted in 44.8 % of cases . Hassan H Ramadan et al[66] 1995 ,Lansford BK et al[67]1995, Anselmo-Lima WT et al [68], 2004,LR Patel et al [69],2004,have also observed hyperglycemia as a significant risk factor for invasive sinusitis. Diabetes causes increased chance of fungal infection because of impaired neutrophil function[17,21]. Diabetes atlas 2011 by International Diabetes Federation has observed that the current number of people affected by Diabetes in India is 61 Million and is expected to rise to 100 Million by 2030 unless preventive measures are taken.They have further predicted that by 2030,one in every 10 adults will have diabetes and 4 in every

5 diabetics will be in the working age group 40-59 years.[103].So , increased vigilance will be needed to identify fungal rhinosinusitis in Diabetes in the future.

Asthma/Chronic Eczema was noted in 46% of other categories(which includes AFS,CGFRS and FB)(Table 5). 16 patients of the 29 patients (55%) who had Allergic fungal sinusitis had given a history of Asthma and /or Chronic eczema. **(p<0.001)**This was found to be statistically significant implying that asthma and other atopic illnesses were significantly associated with AFS. Asthma and associated atopic illnesses were noted in 49.1% of patients with AFS in a study done by Suraiye.H.Al-Dousary etal, 2008[59], 50% by Manning et al [15]and 64% by Schubert et al[63].

19 patients, i.e, 65 % were diagnosed in stage 3 of the disease whereas 34.4% were diagnosed in stage 4(Table 6).This showed that most patients presented to the healthcare facility in fairly advanced stage of the disease. Lildholdt et al [71],1997and Laila .M.Telmisani et al[72] 2009 predicted that the recurrence rate increased significantly with increase in grade of the disease, suggesting that grading system could be used for prediction of recurrence of nasal polyps. Overall recurrence (previous surgery for sinusitis) in our study was 51.7%.Recurrence rate was seen more in stage iii of the disease.11 out of the total 15 recurrent cases (61%) were in the stage iii of the disease. But this association was not statistically significant in our study probably due to loss of follow up of patients. **(p>0.05)**

Direct microscopic (10%) KOH mount examination had a sensitivity of 95.34%.HPE and culture had a sensitivity of 93% and 88.57% respectively. (Table 7) Ravikumar et al [73],2004 and Marple BF et al [74],2002 have shown in their studies that the sensitivity of HPE was 90% .and 85-90% respectively. Though culture is considered as gold standard, culture positivity in this study was 88.37%.A few reasons could be the cause for this discrepancy. Excesssive maceration of the tissue during FESS or during sample processing can result in reduced viability of fungi and also patients already started on antifungals were frequently culture negative[75].Sensitivity will greatly improve if all the methods are combined together.

Most of the isolates were of a single species (81.3%).4.6 % were mixed growth. One of the mixed growth showed both septate and aseptate hyphae in direct KOH mount. The other grew both fungi in both DNE and FESS samples. *Candida spp (C.tropicalis-3 and C.albicans* -3) were isolated in 6 cases.(13.9%).*Candida spp* are known only as colonizers of the sinuses and anterior nares and are known as a very rare cause of sinusitis.

Majority of the fungi isolated were *Aspergillus spp.* in particular *A.flavus(46.5%)(Table 8).A.flavus* was the commonest isolate in AFS(55%),Fungal ball(100%) and Chronic granulomatous disease.(25%).In chronic invasive form, however ,*Rhizopus spp* were the most commmonly isolated(50%).*A.niger* was the 2nd most commonly isolated in AFRS. Manning et al has described 87% of AFRS as due to dematiaceous fungi and the remainder due to *Aspergillus spp*[76].But in India, A.*flavus* is isolated in > 80%

of cases of AFRS[76,77,78,79,81]. This is attributed to the fact that *Aspergillus spp* are the usual fungi present in the soil and environment of tropical countries like India[60]. *Aspergillus species* in high concentrations were cultured from straw roofs,earthen floor and bedding in houses in a study done by Sandison AT [104]Shiv sekar chatterjee et al describes *A.flavus* as the main etiological agent in CGFRS also[6].This parallels the results of our study. In our study *Rhizopus spp*. predominated in the invasive form of the disease. On the contrary, Chakrabarti et al describes *A.fumigatus* as the commonest fungi causing CIFRS[79].

20 isolates of *A.flavus*, 6 of *Rhizopus spp*,4 of *A.fumigatus* ,3 of *A.niger* and 1 each of *A.nidulans, A.clavatus, Penicillium spp*. and *Paecilomyces variotii* were taken up for antifungal susceptibility testing for Amphotericin B and Itraconazole. *ATCC A.flavus* 204304 (generously spared by Dr.Shiv prakash, Institute of Mycology,PGIMER,Chandigarh)was included in each panel as a reference strain.

All the fungal isolates were universally sensitive to both Amphotericin B and Itraconazole by broth microdilution method(Table 9,10).MIC ranged from 0.0313-1 for both the antifungals. The MIC of reference strain was 0.5 and 0.25 for Amphotericin B and Itraconazole respectively which was well within the control limits prescribed by CLSI M38 A[33].

Agar dilution was also done for comparison. The results obtained by agar dilution was comparable to that obtained by microbroth dilution method for all the tested 37 isolates (Table 14).E test(Hi Media,Mumbai) was tried for Amphotericin B.MICs observed were slightly lower than that observed by

broth microdilution method.[25] (Table 13) .By contrast, Meletiadis *et al.* compared the results obtained by the E test with the CLSI document M38-A and found that low levels of agreement between the CLSI and the Etest were found for most species, especially after 48 h of incubation[39]. Martin mazielos et al [40]and Szeley et al[38], Etest MICs of Amphotericin B for *A. flavus* were substantially higher than CLSI MICs[38,40.] Our results correlated if the Etest MICs are recorded after 24 h of incubation rather than 48 h or when sufficient growth is visible as described by Melitiadis et al[39]. But, this study is hampered by the limited number of isolates available for testing. Further research is needed to know the utility of E test for susceptibility testing for filamentous fungi.

Susceptibility testing was also done using Amphotericin B disks 10μg and Itraconazole 10 μg disks(Table 11 and 12). Though percentage of major errors was not much (5%), percentage of minor errors was much higher(20%) for Aspergillus spp by Amphotericin B(Table 15). So, Amphotericin B disk should be used with caution in testing Aspergillus spp. Zygomycetes have given comparable results with broth microdilution and hence Amphotericin B disk could be considered for susceptibility testing of zygomycetes. There was no major errors noted in Itraconazole disks for testing of filamentous fungi and minor error was noted only in insignificant numbers(10%)(Table 16).So, Itraconazole disks can be used for susceptibility testing for filamentous fungi. This is an expanding area of research with a new document released by CLSI quite recently for disk diffusion for moulds and further studies need to be done with a large number of isolates to assess the utility of the same.

The above results are comparable with the studies done by A.Espinell – Ingroff,2007[85], A.Espinell –Ingroff et al,2006[86],lopez Oviedo et al,2006[101] and Serrano MC et al ,2004 [102]who have noted in their respective studies that Itraconazole 10µg disks are suitable for testing of all species except zygomycetes, whereas posoconazole 10µg disks give a better correlation than Itraconazole for testing Zygomycetes. They have further noted that lowest correlation was noted consistently for Amphotericin B Disk 10 µg (23% minor error,3% major error and 1.7% Very Major error noted) and have suggested that Amphotericin B be used for susceptibility testing of only Zygomycetes[85,86].Posaconazole disk diffusion was not done in this study, It could have been included in the study for comparison with Itraconazole disk.

Strong suspicion, meticulous specimen collection & preparation and further studies with a long period of follow up and more number of patients are required to analyse the impact of fungi in the etiopathogenesis of chronic rhinosinusitis.

SUMMARY

- Among the 380 Patients who underwent DNE and/or FESS , 80 patients who complied with the inclusion criteria were included under the study.43 patients were diagnosed as CFRS, contributing to 11.3% of the total cases.

- Of the CFRS categories, **Allergic fungal sinusitis was the most predominant contributing to 67 % of the disease.**

- **Allergic fungal rhinosinusitis** was found more in **young adults(21-40)** ($p<0.05$)and **Invasive fungal sinusitis** was found more in the **middle aged(41-60) .**

- Pansinusitis was the most common presentation in both FRS and non FRS group(58% and 84%).Isolated sinus involvement was observed more commonly in FRS than NFRS. Among the FRS, isolated maxillary, isolated sphenoid and isolated ethmoid fungal sinusitis (14%, 7% and 7% respectively) were commonly observed .

- Nasal block was the most common presentation(90.6%) in CFRS

- **Hyperglycemia** was observed as a statistically significant associated risk factor (75%)for the development of **invasive form of fungal sinusitis** .

- **Asthma and other atopic illnesses were significantly associated(55%) with the development of allergic fungal rhinosinusitis**.

- Among the patients of AFRS, 67% presented late to the health care facility, i.e stage III and Recurrence was noted in 51.7% of AFRS patients.

- **Direct microscopy was highly sensitive** in clinching the diagnosis of CFRS in 95.34% of cases when compared to HPE and culture.

- ***A.flavus* was the most common isolate** in AFRS(55%), FB(100%) and CGFRS(25%) and *Rhizopus spp.* was the most common isolate in CIFRS(50%).

- All isolates were sensitive to Amphotericin B and Itraconazole by broth microdilution method &agar dilution .

- E test can also be used with caution for susceptibility testing of filamentous fungi with meticulous Quality control testing.

- Disk diffusion method with Amphotericin B (10 µg disk) was found to be reliable for susceptibility testing of Zygomycetes as it is easy and suitable for routine testing in laboratories.Similarly,Itraconazole 10µg disk was found to be reliable for susceptibility testing of all filamentous fungi.

- For life threatening invasive fungal infections, broth microdilution must be carried out for Amphotericin B to provide useful information to the clinician even if empirical antifungal therapy is already started for the patient .

CONCLUSION

Chronic Fungal Rhinosinusitis largely impairs the active functioning of the patients in their day to day life and causes a significant morbidity and even mortality. This study was undertaken to assess the prevalence of CFRS, to isolate and identify the fungi &their sensitivity pattern to standard antifungal agents and to study the risk factors favouring CFRS in patients undergoing functional endoscopic sinus surgery and diagnostic nasal endoscopy in Rajiv Gandhi Government general hospital,Chennai. CFRS was noted in 11% of the cases in the present study.

Categorisation of CFRS is essential and helps to decide the best treatment option for the patient. **Allergic fungal sinusitis was the most common presentation noted(67%)**followed by chronic invasive(19%), chronic granulomatous (9%)and fungal ball(5%).Patients with uncontrolled **diabetes are at risk of acquiring invasive form of CFRS**. Patients with documented asthma and associated atopic illnesses are at an increased risk of acquiring AFRS. ***Aspergillus flavus* was most commonly isolated(46.5%)** followed by Rhizopus spp(13.9%), Aspergillus fumigatus(11.6%), A.niger(6.9%), A.clavatus(2.3%), A.versicolor(2.3%), A.nidulans(2.3%), Penicillium spp(2.3%) and Paecilomyces variotii(2.3%). *A.flavus* was the most common isolate in AFRS, FB and CGFRS and *Rhizopus spp.* was the most common isolate in CIFRS. Grave complications like orbital cellulitis, brain abscess, orbital granuloma, cavernous sinus thrombosis and cranial nerve palsies were seen in this study. Direct microscopy was highly sensitive in

clinching the diagnosis of CFRS in 95.34% of cases when compared to HPE and culture. Detection rate increased when direct microscopy and culture were used in conjunction with histopathological examination.

Anti fungal sensitivity testing should be done as a routine for all cases when feasible as resistant strains are emerging.In this study, all isolates were sensitive to Amphotericin B and Itraconazole by broth microdilution method & agar dilution.

Though conventionally, microbroth /agar dilution is done for filamentous fungi, in this study E test and disk diffusion, (**Amphotericin B 10 µg disk for Zygomycetes and Itraconazole 10 µg disk for all filamentous fungi**) were found to be equally good and can be followed for routine testing. But, in life threatening invasive fungal infections, it is prudent to do microbroth/agar dilution for antifungal susceptibility testing.

PROFORMA

S.NO : IP/OP NO.:

Name of the patient: Occupation:

Age: Sex:

Address:

Presenting complaints; Duration

Underlying illness(tick when appropriate):

H/o Asthma

H/o Aspirin allergy

H/o Diabetes mellitus

H/o Chronic Eczema

H/o COPD

H/o Uremia/Chronic Kidney disease

H/o Neoplasm

H/o Immunosuppressive therapy

H/o Faciomaxillary Trauma

H/o previous nose/ throat /sinus surgery

PHYSICAL EXAMINATION:

DNE/FESS:

BIOLOGICAL PARAMETERS:

Blood sugar:

S.urea:

S.creatinine:

OTHER INVESTIGATIONS:

Total WBC count:

Differential count;

Absolute eosinophil count:

X Ray PNS:

CT PNS:

MRI PNS:

Histo pathological examination:

MICROBIOLOGICAL EXAMINATION:

Direct Examination with 10% Potassium hydroxide

CULTURE:

Antifungal susceptibility pattern:

1.Disk diffusion:

2.MIC: By Broth dilution:

By Agar dilution:

By E test:

APPENDIX

I.10% POTASSIUM HYDROXIDE SOLUTION:

Potassium hydroxide-10 gm

Glycerol -10 ml

Distilled water -80 ml

To the solution of 10% KOH, 10% glycerol is added to prevent drying.

The ingredients are mixed and stored at room temperature.

II. Lactophenol cotton blue stain

Lactic acid	20 ml
Phenol	20ml
Cotton blue (dye)	0.5g
Glycerol	40ml
Distilled water	20ml

III.MEDIA USED:

1.SABOURAUD DEXTOSE AGAR WITH ANTIBIOTICS:

COMPOSITION:

Peptone :10 gm

Dextrose :40 gm

Agar :20 gm

Distilled water :1000 ml

Gentamicin :20 mg

Final pH was adjusted to 5.6.

Media preparation:

The above ingredients were reconstituted in one litre of distilled water. Dissolve the powder in distilled water by boiling. Add Gentamicin is added to the boiling medium. The medium was then removed from heating, mixed well and then dispersed in tubes and autoclaved at 121^0 C for 15 minutes and the final pH was adjusted to 5.6.The tubes were cooled in slanted position and later the slants were stored in refrigerator.

2.YEAST NITROGEN BASE AGAR MEDIUM(DEHYDRATED NEDIA, Himedia, MUMBAI)

INGREDIENTS	**GRAMS/L**	**INGREDIENTS**	**GRAMS/L**
Ammonium sulphate	5.00	Thiamine hydrochloride	0.004
L-Histidine	0.01	Boric acid	0.0005
hydrochloride	0.02	Copper sulphate	0.00004
DL-Methionine	0.02	Potassium iodide	0.0001
DL-Tryptophan	0.000002	Ferric chloride	0.0002
Biotin	0.00004	Manganese sulphate	0.0004
Calcium pantothenate	0.000002	Sodium molybdate	0.0002
Folic acid	0.02	Zinc sulphate	0.0004
Inositol	0.0004	Monopotassium	1.00
Niacin	0.0002	phosphate	0.50
Para amino benzoic acid	0.0004	Magnesium sulphate	0.10
Pyridoxine	0.0002	Sodium chloride	0.10
hydrochloride		Calcium chloride	
Riboflavin			

Dissolve 6.7 gms of the media in 100 ml of distilled water. Sterilise by filteration and store at 4^0C.

3.MUELLER HINTON AGAR:

Beef infusion : 300 ml

Casein hydrosylate : 17.5 gm

Starch : 1.5 gm

Agar : 10 gm

Distilled Water : 1000 ml

pH : 7.4

Sterilize by autoclaving at 121^0C for 20 minutes.

4.RPMI 1640(ROSEWALL PARK MEMORIAL INSTITUTE) MEDIA:

* RPMI medium : 10.4 gm

* MOPS buffer :34.43 gm

Dissolve powdered medium in 900 ml distilled water . Add MOPS to a final concentration of 0.165 mol/ L and stir until dissolved. While stirring, adjust the pH to 7.0 at 25^0 C. Add additional water to bring medium to a final volume of 1000 ml. Filter sterilize and store at 4^0C.

5. POTATO DEXTROSE AGAR:

Potato :200 G Dextrose:20 G

Agar : 20 G

Water : 1 Litre

Boil 200 g of potatoes in 1 litre of water for 60 minutes. Squeeze as much as pulp as possible through a fine sieve. Add agar and boil till it dissolves.Add dextrose and make upto 1 litre. Dispense in required amounts taking care to keep the solids in suspension. Autoclave at 115^0C for 30 minutes. Cool to 50^0C and pour into petridishes.

ABBREVIATIONS

AFRS	:	Allergic Fungal Rhinosinusitis
CFRS	:	Chronic Fungal Rhinosinusitis
CGFRS	:	Chronic Granulomatous Fungal Rhinosinusitis
CIFRS	:	Chronic Invasive Fungal Rhinosinusitis
CLSI	:	Clinical Laboratory Standard Institute
DMSO	:	Dimethyl sulfoxide
E TEST	:	Epsilometer test
ELISA	:	Enzyme Linked Immunosorbent Assay
FB	:	Fungal ball
FRS	:	Fungal Rhinosinusitis
GMS	:	Gomori Methenamine silver
H%E	:	Haematoxylin and eosin
HPE	:	Histopathological examination
KOH	:	Potassium hydroxide mount
m	:	Minor error

M	:	Major error
VM	:	Very Major error
MHA	:	Mueller Hinton Agar
MIC	:	Minimum Inhibitory Concentration
MOPS	:	3N-Morpholino propane sulphonic acid
MRI	:	Magnetic resonance imaging
NFRS	:	Non fungal rhinosinusitis
PAS	:	Periodic acid schiff
PDA	:	Potato Dextrose agar
PNS	:	Paranasal sinus
RPMI	:	Rosewall Park Memorial Institute
R	:	Resistant
S	:	Susceptible
SDD	:	Susceptible Dose Dependent

CONSENT FORM

STUDY TITLE:

A STUDY ON THE MYCOLOGICAL PROFILE, CATEGORIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CHRONIC FUNGAL RHINOSINUSITIS IN A TERTIARY CARE HOSPITAL

STUDY CENTRE:

Institute of microbiology, Rajiv Gandhi govt. General hospital, Chennai-03.

Name: Date:

Age/Sex: IP.NO:

I conform that I understand the purpose of the above study and I have the opportunity to ask questions. All my questions and doubts have been answered to my satisfaction. I understand that my participation in this study is voluntary and I am free to withdraw at any time without giving any reason. I understand that the investigator, regulatory authorities and ethical committees will not need my permission, to look at my health records both in respect to current study and any further research that be conducted in relation to it.

I understand that my participation in the study will not affect my treatment. I have received information sheet regarding the research.I hereby consent to participate in study "A STUDY ON THE MYCOLOGICAL PROFILE, CATEGORIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CHRONIC FUNGAL RHINOSINUSITIS IN A TERTIARY CARE HOSPITAL" conducted at Institute of microbiology, Rajiv Gandhi govt. General hospital, Chennai-03.

Date:

Place: Signature/thumb impression of patient

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MASTER CHART

name	age	type	s/m	site	etiology	m/f	risk factore		stge	sympto ms	KOH	HPE	Ampho s(br, agar ,etest)	Ampho S (disk)	itra S(broth, agar dilution)	itra S(disk)
yuvarani	21	all	single	pan	a.flavus	f	asthma	recurrence	stage iii	1,2,4,	koh +	hpe +	s	S	s	S
barathy	27	proven invasive	single	pan	a.niduulans	f	diabetes			1,2,5,8	koh +	hpe +	s	s	s	s
abinesh	20	nfrs		pan		m				2,3,4,5						
mangalam23	23	nfrs		pan		f	ckd			1,2,3,5						
kavitha	23	all	single	pan	a.flavus	f		recurrence	stage iii	1,2,5	koh +	hpe +	s	R	s	S
ajithkumar	24	nfrs		pan		m				1,2,3,						
sureshwari	27	all	single	pan	a.flavus	f		recurrence	stage iii	1,2,,4,	koh +	hpe +	s	S	s	S
sudhakar	30	proven invasive	single	pan	a.clavatus	m	diabetes			1,3,5,9	koh +	hpe +	s	s	s	s
kishore	20	all	single	pan	ng	m	asthma		stage iii	1,3,5	koh +	hpe +	-			
rahulgandhi	21	all	mixed	b/l ethmoid	a.niger+rhizopus	m	ckd/ns	recurrence	stage ii	1,2,3,5	koh +	hpe +	s	s	s	s
karthikayan	25	nfrs		pan		m				2,3,4,5						
ganesh	28	nfrs		pan		m	ckd			1,2,3,						
bhuvaneshwari	35	nfrs		pan		f				2,3,4,5						
poongodi	38	cg	single	right maxilla	rhizopus	f				1,2,3,5	koh +	hpe +	s	sdd	s	s
karpagavalli	38	nfrs		right maxilla		f				1,2,4,5						
bakiyalakshmi	28	all	single	pan	a.niger	f			stage iii	1,3,4,	koh +	hpe +	s	s	s	s
gowri	44	fb	single	left, maxilla	a.flavus	f				2,3	koh +	hpe +	s	S	s	S
shanmugam	40	nfrs		pan		m				1,2,3,4,						
kalyanasundaram	45	proven invasive	single	pan	rhizopus	m	diabetes, renal tr			1,2,4,5	koh +	hpe +	s	s	s	s
alamelu	47	probable invasive		pan, orbit	ng	f		recurrence		1,2,6,9	koh +	hpe +				
sathya	40	nfrs		pan		f	diabetes			2,3,4,5						

dilip kumar	28	cg	single	rightmaxilla	a.fumi	m				2,3	koh +	hpe +	s	S	S	SDD
latha	30	all	single	pan	a.flavus	f		recurrence	stage iii	1,3,,5	koh +	hpe +	s	S	s	S
jayaraman	60	cg	single	left,maxilla	a.versicolor	m				2,3,5	koh +	hpe -	s	s	s	s
annalakshmi	30	all	single	pan	a.flavus	f			stage ii	1,2,4,5	koh +	hpe +	s	S	s	S
violet	32	all	single	pan	a.flavus	f	asthma		stage iii	1,3,5	koh +	hpe +	s	sdd	s	S
venkatesan	21	all	single	pan	a.flavus	m	asthma	recurrence	stage iii	3,4	koh +	hpe +	s	S	s	SDD
anandhi	40	nfrs		pan		f				1,2,3,						
boopalan	50	probable invasive		pan,orbit	ng	m	diabetes	recurrence		1,3,6,7	koh +	hpe +				
vimala	40	nfrs		pan		f				1,2,3,4,						
velu	21	all	single	b/l maxilla	a.flavus	m	jna operated	recurrence	stage iii	1,3,4,5	koh -	hpe -	s	R	s	S
saraswathi	42	nfrs		pan		f				1,2,3,4,						
rajalakshmi	32	all	single	pan	a.flavus	f	asthma		stage iii	1,2,4,5	koh +	hpe +	s	R	s	S
sandeep	26	all	single	b/l maxilla	a.flavus	m	diabetes, asthma	recurrence	stage ii	1,2,5	koh +	hpe +	s	R	s	S
menaga	45	fb	single	left,maxilla	a.flavus	f				1,2,3,5	koh +	hpe +	s	R	s	SDD
annalakshmi	33	all	single	pan	a.flavus	f			stage iii	1,2,3,5	koh +	hpe +	s	R	s	S
poonkundram	26	all		b/l sphenoid	a.fumi	m	asthma		stage ii	1,3,4,5	koh +	hpe +	s	s	s	s
poornachandra	26	all	single	pan	a.flavus	m	asthma	recurrence	stage iii	1,2,3,5	koh +	hpe +	s	R	s	S
angyammal	42	nfrs		pan		f				1,2,3,4,5						
jeyaraman	43	cg	single	left,maxilla orbit	a.flavus	m				1,2,4,5	koh +	hpe +	s	S	s	S
mahesh	45	all	single	b/l ethmoid	paecilomyces	m			stage iii	1,3,4,5	koh +	hpe +	s	s	s	s
ramadevi	33	all	single	pan	a.flavus	f	asthma		stage iii	1,2,4,5	koh +	hpe +	s	R	s	S
selvaraj	45	nfrs		pan		m				1,2,3,4,5						

nasumm	52	all		b/l maxilla	ng	m	asthma	recurrence	stage iii	1,2,4,5	koh +	hpe +				
kuppammal	45	nfrs		pan		f	diabetes			2,3,4,5						
rani	46	all	single	b/l sphenoid	a.flavus	f		recurrence	stage ii	1,2,5	koh +	hpe +	s	S	s	S
anthony pappa	45	nfrs		pan		f				1,3,4,5						
adhilakshmi	48	all	single	b/l maxilla	a.flavus	f	asthma		stage iii	1,3,	koh +	hpe +	s	sdd	s	S
narayanan	45	nfrs		pan		m				1,3,4,5						
murugessan	46	nfrs		pan		m				1,2,3,4						
arundavamary	55	all	single	pan	a.fumi	f	asthma	recurrence	stage iii	1,3,4,	koh +	hpe +	s	S	S	S
swaminathan	47	nfrs		pan		m				1,2,3,5						
vasanthi	48	nfrs		pan		f	asthma			1,2,3,						
muniammal	48	nfrs		rightmaxilla		f				1,2,3,						
egambaram	49	nfrs		pan		m				,2,3,4,5						
devaki	56	all	single	pan	a.flavus	f	asthma	recurrence	stage ii	1,2,5	koh +	hpe +	s	S	s	S
hairunsaral	59	all	single	pan	penicillium	f			stage ii	1,2,5	koh +	hpe +	s	s	s	s
mariyamma	49	nfrs		pan		f				,2,3,4,5						
narasimhan	56	all	single	b/l sphenoid	a.niger	m			stage ii	1,2,4,	kon -	hpe +	s	s	s	s
kannan	50	nfrs		pan		m	asthma			1,2,3,5						
anandhi	63	all		b/l ethmoid	ng	f	asthma		stage iii	1,3,5,	koh +	hpe +				
muniammal	53	proven invasive	single	b/l maxilla	rhizopus	f	diabetes			1,2,3,	Koh +	hpe +	s	s	s	s
jeyabalan	50	nfrs		b/l maxilla		m				1,2,3,4,5						
rangaraj	55	nfrs		pan		m				2,3,4,5						
kasthuri	55	nfrs		pan		f				2,3,4,5						
arulmozhi	56	nfrs		b/l maxilla		f				2,3,4,5						
raji	58	proven invasive	single	b/l maxilla	rhizopus	m			stage iii	1,2,5	koh +	hpe +	s	s	s	s

gangaiammal	60	nfrs		rightmaxilla		f				1,3,4,5						
govindan	60	nfrs		pan		m				1,2,3,5						
kamala	60	nfrs		pan		m										
kasi	61	nfrs		pan		m				1,2,3,4,						
selvaraj	62	nfrs		pan		m	asthma			1,3,4,5						
vincent	64	nfrs		pan		m				1,2,3,						
saraadabai	65	nfrs		pan		f	diabetes			1,2,3,						
mangalakshmi	70	all		pan	ng	f	diabetes, asthma		stage ii	1,2,4,5	koh +	hpe -				
sambasivam	66	nfrs		pan		m				1,2,3,5						
thirasammal	75	nfrs		b/l maxilla		f				,2,3,4,5						
kamalakannan	53/m	proven invasive	mixed	pan,orbit	a.fumi,rhizo	m	diabetes, ckd			1,3,5,6,7,9	koh +	hpe +	S	S	S	S

1-nasal block
2-nasal discharge
3-headache
4-facial puffiness
5-proptosis
6:anosmia
7-tinnitus
8-RRTI
9-OTHERS

A STUDY ON THE MYCOLOGICAL PROFILE, CATEGORIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CHRONIC FUNGAL RHINOSINUSITIS IN A TERTIARY CARE HOSPITAL

ABSTRACT

Fungal sinusitis is being increasingly recognized in persons of all age groups, resulting in great socioeconomic effects. This study was conducted in a tertiary care hospital to evaluate the occurrence of Chronic fungal rhinosinusitis in patients admitted with a radiological diagnosis of rhinosinusitis and undergoing diagnostic and therapeutic endoscopic procedures for the same in atertiary care hospital.

AIM:

This study was conducted with the aim to isolate and identify the fungi causing chronic fungal rhinosinusitis,to categorise the types of fungal sinusitis,to assess the risk factors favouring fungal involvement of paranasal sinuses ,to study the susceptibility pattern of the fungal isolates to standard anti fungal drugs and to compare different methods of susceptibility testing for the fungal isolates.

MATERIALS AND METHODS :

Sample collection,processing and identification of the fungal isolates were done following standard operating procedures.Antifungal susceptibility testing was done by Broth microdilution,Agar dilution ,Disk diffusion and E test and the results compared.

RESULTS AND DISCUSSION:

CFRS was noted in 11% of the cases in the present study.Allergic fungal sinusitis was the most common presentation noted(67%)followed by chronic invasive(19%) ,chronic granulomatous (9%)and fungal ball(5%).Patients with uncontrolled diabetes are at risk of acquiring invasive form of CFRS. Patients with documented asthma and associated atopic illnesses are at an increased risk of acquiring AFRS. *Aspergillus flavus* was most commonly isolated(46.5%) . *A.flavus* was the most common isolate in AFRS, FB and CGFRS and *Rhizopus spp.* was the most common isolate in CIFRS. All isolates were sensitive to Amphotericin B and Itraconazole by broth microdilution method &agar dilution .E test can also be used with caution for susceptibility testing of filamentous fungi with meticulous Quality control testing.Disk diffusion method with Amphotericin B (10 µg disk) was found to be reliable for susceptibility testing of Zygomycetes as it is easy and suitable for routine testing in laboratories.Similarly,Itraconazole 10µg disk was found to be reliable for susceptibility testing of all filamentous fungi.

CONCLUSION:

CFRS was noted in 11% of the cases in the present study. Categorisation of CFRS is essential and helps to decide the best treatment option for the patient. *Aspergillus flavus* was most commonly isolated .Anti fungal sensitivity testing should be done as a routine for all cases when feasible as resistant strains are emerging E test and disk diffusion, (Amphotericin B 10 µg disk for Zygomycetes and Itraconazole 10 µg disk for all filamentous fungi) were found to be equally good and can be followed for routine testing. But, in life threatening invasive fungal infections, it is prudent to do microbroth/agar dilution for antifungal susceptibility testing.

KEY WORDS: Fungal sinusitis, Categories, Susceptibility testing